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(54) Title: COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIALINFECTION

(57) Abstract

Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a *Chlamydia* antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

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COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION

TECHNIGAL FIELD

The present invention relates generally to the detection and treatment of Chlamydial infection. In particular, the invention is related to polypeptides comprising a *Chlamydia* antigen and the use of such polypeptides for the serodiagnosis and treatment of Chlamydial infection.

BACKGROUND OF THE INVENTION

Chlamydiae are intracellular bacterial pathogens that are responsible for a wide variety of important human and animal infections. Chlamydia trachomatis is one of the most common causes of sexually transmitted diseases and can lead to pelvic inflammatory disease (PID), resulting in tubal obstruction and infertility. Chlamydia trachomatis may also play a role in male infertility. In 1990, the cost of treating PID in the US was estimated to be \$4 billion. Trachoma, due to ocular infection with Chlamydia trachomatis, is the leading cause of preventable blindness worldwide. Chlamydia pneumonia is a major cause of acute respiratory tract infections in humans and is also believed to play a role in the pathogenesis of atherosclerosis and, in particular, coronary heart disease. Individuals with a high titer of antibodies to Chlamydia pneumonia have been shown to be at least twice as likely to suffer from coronary heart disease as seronegative individuals. Chlamydial infections thus constitute a significant health problem both in the US and worldwide.

Chlamydial infection is often asymptomatic. For example, by the time a woman seeks medical attention for PID, irreversible damage may have already occurred resulting in infertility. There thus remains a need in the art for improved vaccines and pharmaceutical

compositions for the prevention and treatment of *Chlamydia* infections. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for the diagnosis and therapy of *Chlamydia* infection. In one aspect, the present invention provides polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, or a variant of such an antigen. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments,, the polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of (a) a sequence of SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290; (b) the complements of said sequences; and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions. In specific embodiments, the polypeptides of the present invention comprise at least a portion of a *Chlamydial* protein that includes an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 5-14, 17-20, 26, 28, 30-32, 34, 39-43, 65, 89-109, 138-158, 167, 168, 224-262, 246, 247, 254-256, 292, and variants thereof.

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a *Chlamydial* protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

In a related aspect, polynucleotide sequences encoding the above polypeptides, recombinant expression vectors comprising one or more of these polynucleotide sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides fusion proteins comprising an inventive polypeptide, or, alternatively, an inventive polypeptide and a known *Chlamydia* antigen, as well as polynucleotides encoding such fusion proteins, in combination with a physiologically acceptable carrier or immunostimulant for use as pharmaceutical compositions and vaccines thereof.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody, both polyclonal and monoclonal, or antigen-binding fragment thereof that specifically binds to a *Chlamydial* protein; and (b) a physiologically acceptable carrier. Within other aspects, the present invention provides pharmaceutical compositions that comprise one or more *Chlamydia* polypeptides disclosed herein, or a polynucleotide molecule encoding such a polypeptide, and a physiologically acceptable carrier. The invention also provides vaccines for prophylactic and therapeutic purposes comprising one or more of the disclosed polypeptides and an immunostimulant, as defined herein, together with vaccines comprising one or more polynucleotide sequences encoding such polypeptides and an immunostimulant.

In yet another aspect, methods are provided for inducing protective immunity in a patient, comprising administering to a patient an effective amount of one or more of the above pharmaceutical compositions or vaccines.

In yet a further aspect, methods for the treatment of Chlamydia infection in a patient are provided, the methods comprising obtaining peripheral blood mononuclear cells (PBMC) from the patient, incubating the PBMC with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated T cells and administering the incubated T cells to the patient. The present invention additionally provides methods for the treatment of Chlamydia infection that comprise incubating antigen presenting cells with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated antigen presenting cells and administering the incubated antigen presenting cells to the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient. In certain embodiments, the antigen presenting cells are selected from the group consisting of dendritic cells, macrophages, monocytes, Bcells, and fibroblasts. Compositions for the treatment of Chlamydia infection comprising T cells or antigen presenting cells that have been incubated with a polypeptide or polynucleotide of the present invention are also provided. Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, within other aspects, methods for

removing *Chlamydial*-infected cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a *Chlamydial* protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of *Chlamydial* infection in a patient, comprising administering to a patient a biological sample treated as described above. In further aspects of the subject invention, methods and diagnostic kits are provided for detecting *Chlamydia* infection in a patient. In one embodiment, the method comprises: (a) contacting a biological sample with at least one of the polypeptides or fusion proteins disclosed herein; and (b) detecting in the sample the presence of binding agents that bind to the polypeptide or fusion protein, thereby detecting *Chlamydia* infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. In one embodiment, the diagnostic kits comprise one or more of the polypeptides or fusion proteins disclosed herein in combination with a detection reagent. In yet another embodiment, the diagnostic kits comprise either a monoclonal antibody or a polyclonal antibody that binds with a polypeptide of the present invention.

The present invention also provides methods for detecting *Chlamydia* infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous nucleotides of a polynucleotide sequence peptide disclosed herein, or of a sequence that hybridizes thereto.

In a further aspect, the present invention provides a method for detecting Chlamydia infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe. In one embodiment, the oligonucleotide probe

comprises at least about 15 contiguous nucleotides of a polynucleotide sequence disclosed herein, or a sequence that hybridizes thereto.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

SEQUENCE IDENTIFIERS

SEQ ID NO: 1 is the determined DNA sequence for the C. trachomatis clone

1-B1-66.

SEQ ID NO: 2 is the determined DNA sequence for the C. trachomatis clone

4-D7-28.

SEQ ID NO: 3 is the determined DNA sequence for the C. trachomatis clone

3-G3-10.

SEQ ID NO: 4 is the determined DNA sequence for the C. trachomatis clone

10-C10-31.

SEQ ID NO: 5 is the predicted amino acid sequence for 1-B1-66.

SEQ ID NO: 6 is the predicted amino acid sequence for 4-D7-28.

SEQ ID NO: 7 is a first predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 8 is a second predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 9 is a third predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 10 is a fourth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 11 is a fifth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 12 is the predicted amino acid sequence for 10-C10-31.

SEQ ID NO: 13 is the amino acid sequence of the synthetic peptide 1-B1-

66/48-67.

SEQ ID NO: 14 is the amino acid sequence of the synthetic peptide 1-B1-

66/58-77.

SEQ ID NO: 15 is the determined DNA sequence for the C. trachomatis serovar LGV II clone 2C7-8

SEQ ID NO: 16 is the determined DNA sequence for a first putative open reading frame from C. trachomatis serovar D

SEQ ID NO: 17 is the predicted amino acid sequence encoded by the first putative open reading frame from C. trachomatis serovar D

SEQ ID NO: 18 is the amino acid sequence of the synthetic peptide CtC7.8-12

SEQ ID NO: 19 is the amino acid sequence of the synthetic peptide CtC7.8-13

SEQ ID NO: 20 is the predicted amino acid sequence encoded by a second putative open reading from C. trachomatis serovar D

SEQ ID NO: 21 is the determined DNA sequence for clone 4C9-18 from C. trachomatis LGV II

SEQ ID NO: 22 is the determined DNA sequence homologous to Lipoamide Dehydrogenase from *C. trachomatis* LGV II

SEQ ID NO: 23 is the determined DNA sequence homologous to Hypothetical protein from C. trachomatis LGV II

SEQ ID NO: 24 is the determined DNA sequence homologous to Ubiquinone Mehtyltransferase from C. trachomatis LGV II

SEQ ID NO: 25 is the determined DNA sequence for clone 4C9-18#2 BL21 pLysS from C. trachomatis LGV II

SEQ ID NO: 26 is the predicted amino acid sequence for 4C9-18#2 from C. trachomatis LGV II

SEQ ID NO: 27 is the determined DNA sequence for Cp-SWIB from C. pneumonia strain TWAR

SEQ ID NO: 28 is the predicted amino acid sequence for Cp-SWIB from C. pneumonia strain TWAR

SEQ ID NO: 29 is the determined DNA sequence for Cp-S13 from C. pneumonia strain TWAR

SEQ ID NO: 30 is the predicted amino acid sequence for Cp-S13 from C. pneumonia strain TWAR

SEQ ID NO: 31 is the amino acid sequence for a 10mer consensus peptide from CtC7.8-12 and CtC7.8-13

SEQ ID NO: 32 is the predicted amino acid sequence for clone 2C7-8 from C. trachomatis LGV II

SEQ ID NO: 33 is the determined DNA sequence of a clone from C. trachomatis serovar D which shows homology to clone 2C7-8

SEQ ID NO: 34 is the predicted amino acid sequence encoded by the sequence of SEQ ID NO: 33

SEQ ID NO: 35 is the DNA sequence for C.p. SWIB Nde (5' primer) from C. pneumonia

SEQ ID NO: 36 is the DNA sequence for C.p. SWIB EcoRI (3' primer) from C. pneumonia

SEQ ID NO: 37 is the DNA sequence for C.p. S13 Nde (5' primer) from C. pneumonia

SEQ ID NO: 38 is the DNA sequence for C.p. S13 EcoRI (3' primer) from C. pneumonia

SEQ ID NO: 39 is the amino acid sequence for CtSwib 52-67 peptide from C. trachomatis LGV II

SEQ ID NO: 40 is the amino acid sequence for CpSwib 53-68 peptide from C. pneumonia

SEQ ID NO: 41 is the amino acid sequence for HuSwib 288-302 peptide from Human SWI domain

SEQ ID NO: 42 is the amino acid sequence for CtSWI-T 822-837 peptide from the topoisomerase-SWIB fusion of *C. trachomatis*

SEQ ID NO: 43 is the amino acid sequence for CpSWI-T 828-842 peptide from the topoisomerase-SWIB fusion of *C. pneumonia*

SEQ ID NO: 44 is a first determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II clone</u> 19783.3,jen.seq(1>509)CTL2#11-3', representing the 3' end.

SEQ ID NO: 45 is a second determined DNA sequence for the *C. trachomatis* LGV II clone 19783.4,jen.seq(1>481)CTL2#11-5', representing the 5' end.

SEQ ID NO: 46 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II clone</u>19784CTL2_12consensus.seq(1>427)CTL2#12.

SEQ ID NO: 47 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19785.4,jen.seq(1>600)CTL2#16-5', representing the 5' end.

SEQ ID NO: 48 is a first determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II clone</u> 19786.3,jen.seq(1>600)CTL2#18-3', representing the 3' end.

SEQ ID NO: 49 is a second determined DNA sequence for the *C. trachomatis* LGV II clone 19786.4,jen.seq(1>600)CTL2#18-5', representing the 5' end.

SEQ ID NO: 50 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19788CTL2_21consensus.seq(1>406)CTL2#21.

SEQ ID NO: 51 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19790CTL2_23consensus.seq(1>602)CTL2#23.

SEQ ID NO: 52 is the determined DNA sequence for the <u>C. trachomatis LGV</u>
<u>II clone</u> 19791CTL2_24consensus.seq(1>145)CTL2#24.

SEQ ID NO: 53 is the determined DNA sequence for the *C. trachomatis* LGV II clone CTL2#4.

SEQ ID NO: 54 is the determined DNA sequence for the *C. trachomatis* LGV II clone CTL2#8b.

SEQ ID NO: 55 is the determined DNA sequence for the *C. trachomatis* LGV II clone15-G1-89, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 56 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II clone 14-H1-4</u>, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 57 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-G3-83, sharing homology to the hypothetical protein CT622.

SEQ ID NO: 58 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-B3-95, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 59 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-H4-28, sharing homology to the dnaK gene CT396.

SEQ ID NO: 60 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 11-H3-68, sharing partial homology to the PGP6-D virulence protein and L1 ribosomal gene CT318.

SEQ ID NO: 61 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-G1-34, sharing partial homology to the malate dehydrogenase gene CT376 and to the glycogen hydrolase gene CT042.

SEQ ID NO: 62 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-G10-46, sharing homology to the hypothetical protein CT610.

SEQ ID NO: 63 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-C12-91, sharing homology to the OMP2 gene CT443.

SEQ ID NO: 64 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II. clone 11-A3-93</u>, sharing homology to the HAD superfamily gene CT103.

SEQ ID NO: 65 is the determined amino acid sequence for the *C. trachomatis*<u>LGV II</u> clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 66 is the determined DNA sequence for the *C. trachomatis* LGV II clone CtL2#9.

SEQ ID NO: 67 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone CtL2#7.

SEQ ID NO: 68 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone CtL2#6.

SEQ ID NO: 69 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone CtL2#5.

SEQ ID NO: 70 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone CtL2#2.

SEQ ID NO: 71 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone CtL2#1.

SEQ ID NO: 72 is a first determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 23509.2CtL2#3-5', representing the 5' end.

SEQ ID NO: 73 is a second determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 23509.1CtL2#3-3', representing the 3' end.

SEQ ID NO: 74 is a first determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 22121.2CtL2#10-5', representing the 5' end.

SEQ ID NO: 75 is a second determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 22121.1CtL2#10-3', representing the 3' end.

SEQ ID NO: 76 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 19787.6CtL2#19-5', representing the 5' end.

SEQ ID NO: 77 is the determined DNA sequence for the <u>C. pneumoniae LGV</u> <u>II</u> clone CpS13-His.

SEQ ID NO: 78 is the determined DNA sequence for the <u>C. pneumoniae LGV</u> <u>II</u> clone Cp_SWIB-His.

SEQ ID NO: 79 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 23-G7-68, sharing partial homology to the L11, L10 and L1 ribosomal protein.

SEQ ID NO: 80 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 22-F8-91, sharing homology to the pmpC gene.

SEQ ID NO: 81 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 21-E8-95, sharing homology to the CT610-CT613 genes.

SEQ ID NO: 82 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 19-F12-57, sharing homology to the CT858 and recA genes.

SEQ ID NO: 83 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 19-F12-53, sharing homology to the CT445 gene encoding glutamyl tRNA synthetase.

SEQ ID NO: 84 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 19-A5-54, sharing homology to the cryptic plasmid gene.

SEQ ID NO: 85 is the determined DNA sequence for the <u>C. trachomatis LGV</u>
<u>II</u> clone 17-E11-72, sharing partial homology to the OppC_2 and pmpD genes.

SEQ ID NO: 86 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 17-C1-77, sharing partial homology to the CT857 and CT858 open reading frames.

SEQ ID NO: 87 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 15-H2-76, sharing partial homology to the pmpD and SycE genes, and to the CT089 ORF.

SEQ ID NO: 88 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 15-A3-26, sharing homology to the CT858 ORF.

SEQ ID NO: 89 is the determined amino acid sequence for the <u>C. pnuemoniae</u> clone Cp_SWIB-His.

SEQ ID NO: 90 is the determined amino acid sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone CtL2_LPDA_FL.

SEQ ID NO: 91 is the determined amino acid sequence for the $\underline{C.\ pnuemoniae}$ clone CpS13-His.

SEQ ID NO: 92 is the determined amino acid sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone CtL2_TSA_FL.

SEQ ID NO: 93 is the amino acid sequence for Ct-Swib 43-61 peptide from C. trachomatis LGV II.

SEQ ID NO: 94 is the amino acid sequence for Ct-Swib 48-67 peptide from C. trachomatis LGV II.

SEQ ID NO: 95 is the amino acid sequence for Ct-Swib 52-71 peptide from C. trachomatis LGV II.

SEQ ID NO: 96 is the amino acid sequence for Ct-Swib 58-77 peptide from *C. trachomatis* LGV II.

SEQ ID NO: 97 is the amino acid sequence for Ct-Swib 63-82 peptide from *C. trachomatis* LGV II.

SEQ ID NO: 98 is the amino acid sequence for Ct-Swib 51-66 peptide from C. trachomatis LGV II.

SEQ ID NO: 99 is the amino acid sequence for Cp-Swib 52-67 peptide from C. pneumonia.

SEQ ID NO: 100 is the amino acid sequence for Cp-Swib 37-51 peptide from C. pneumonia.

SEQ ID NO: 101 is the amino acid sequence for Cp-Swib 32-51 peptide from C. pneumonia.

SEQ ID NO: 102 is the amino acid sequence for Cp-Swib 37-56 peptide from C. pneumonia.

SEQ ID NO: 103 is the amino acid sequence for Ct-Swib 36-50 peptide from C. trachomatis.

SEQ ID NO: 104 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis.

SEQ ID NO: 105 is the amino acid sequence for Ct-S13 60-80 peptide from C. trachomatis.

SEQ ID NO: 106 is the amino acid sequence for Ct-S13 1-20 peptide from C. trachomatis.

SEQ ID NO: 107 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis.

SEQ ID NO: 108 is the amino acid sequence for Ct-S13 56-75 peptide from *C. trachomatis*.

SEQ ID NO: 109 is the amino acid sequence for Cp-S13 56-75 peptide from *C. pneumoniae*.

SEQ ID NO: 110 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 21-G12-60, containing partial open reading frames for hypothetical proteins CT875, CT229 and CT228.

SEQ ID NO: 111 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 22-B3-53, sharing homology to the CT110 ORF of GroEL.

SEQ ID NO: 112 is the determined DNA sequence for the *C. trachomatis* LGV II clone 22-A1-49, sharing partial homology to the CT660 and CT659 ORFs.

SEQ ID NO: 113 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 17-E2-9, sharing partial homology to the CT611 and CT 610 ORFs.

SEQ ID NO: 114 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 17-C10-31, sharing partial homology to the CT858 ORF.

SEQ ID NO: 115 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 21-C7-66, sharing homology to the dnaK-like gene.

SEQ ID NO: 116 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 20-G3-45, containing part of the pmpB gene CT413.

SEQ ID NO: 117 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 18-C5-2, sharing homology to the S1 ribosomal protein ORF.

SEQ ID NO: 118 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 17-C5-19, containing part of the ORFs for CT431 and CT430.

SEQ ID NO: 119 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 16-D4-22, contains partial sequences of ORF3 and ORF4 of the plasmid for growth within mammalian cells.

SEQ ID NO: 120 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar LGV II</u> Cap1 gene CT529.

SEQ ID NO: 121 is the predicted full-length amino acid sequence for the \underline{C} . $\underline{trachomatis}$ serovar LGV II Cap1 gene CT529.

SEQ ID NO: 122 is the determined full-length DNA sequence for the \underline{C} . $\underline{trachomatis}$ serovar \underline{E} Cap1 gene CT529.

SEQ ID NO: 123 is the predicted full-length amino acid sequence for the \underline{C} . $\underline{trachomatis}$ serovar \underline{E} Cap1 gene CT529.

SEQ ID NO: 124 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar 1A</u> Cap1 gene CT529.

SEQ ID NO: 125 is the predicted full-length amino acid sequence for the \underline{C} . $\underline{trachomatis}$ serovar 1A Cap1 gene CT529.

SEQ ID NO: 126 is the determined full-length DNA sequence for the \underline{C} . $\underline{trachomatis}$ serovar G Cap1 gene CT529.

SEQ ID NO: 127 is the predicted full-length amino acid sequence for the \underline{C} . $\underline{trachomatis}$ serovar \underline{G} Cap1 gene CT529.

SEQ ID NO: 128 is the determined full-length DNA sequence for the \underline{C} . $\underline{trachomatis}$ serovar F1 NII Cap1 gene CT529.

SEQ ID NO: 129 is the predicted full-length amino acid sequence for the \underline{C} . $\underline{trachomatis}$ serovar F1 NII Cap1 gene CT529.

SEQ ID NO: 130 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar L1</u> Cap1 gene CT529.

SEQ ID NO: 131 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar L1</u> Cap1 gene CT529.

SEQ ID NO: 132 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar L3</u> Cap1 gene CT529.

SEQ ID NO: 133 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar L3 Cap1</u> gene CT529.

SEQ ID NO: 134 is the determined full-length DNA sequence for the \underline{C} . $\underline{trachomatis}$ serovar Ba Cap1 gene CT529.

SEQ ID NO: 135 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar Ba</u> Cap1 gene CT529.

SEQ ID NO: 136 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar MOPN</u> Cap1 gene CT529.

SEQ ID NO: 137 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar MOPN</u> Cap1 gene CT529.

SEQ ID NO: 138 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #124-139 of *C. trachomatis* serovar L2.

SEQ ID NO: 139 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #132-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 140 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-155 of *C. trachomatis* serovar L2.

SEQ ID NO: 141 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #146-163 of C. trachomatis serovar L2.

SEQ ID NO: 142 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #154-171 of *C. trachomatis* serovar L2.

SEQ ID NO: 143 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #162-178 of C. trachomatis serovar L2.

SEQ ID NO: 144 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-147 of C. trachomatis serovar L2.

SEQ ID NO: 145 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #139-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 146 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #140-147 of C. trachomatis serovar L2.

SEQ ID NO: 147 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-146 of *C. trachomatis* serovar L2.

SEQ ID NO: 148 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-145 of C. trachomatis serovar L2.

SEQ ID NO: 149 is the determined amino acid sequence for the Cap1 CT529 ORF peptide # F140->I of C. trachomatis serovar L2.

SEQ ID NO: 150 is the determined amino acid sequence for the Cap1 CT529 ORF peptide ##S139>Ga of C. trachomatis serovar L2.

SEQ ID NO: 151 is the determined amino acid sequence for the Cap1 CT529 ORF peptide ##S139>Gb of C. trachomatis serovar L2.

SEQ ID NO: 152 is the determined amino acid sequence for the peptide # 2 C7.8-6 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 153 is the determined amino acid sequence for the peptide # 2 C7.8-7 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 154 is the determined amino acid sequence for the peptide # 2 C7.8-8 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 155 is the determined amino acid sequence for the peptide # 2 C7.8-9 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 156 is the determined amino acid sequence for the peptide # 2 C7.8-10 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 157 is the determined amino acid sequence for the 53 amino acid residue peptide of the 216aa ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 158 is the determined amino acid sequence for the 52 amino acid residue peptide of the CT529 ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 159 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 160 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 161 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 for serovars other than L2 and MOPN.

SEQ ID NO: 162 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovars other than L2 and MOPN.

SEQ ID NO: 163 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 164 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 165 is the determined DNA sequence for the 5' (forward) primer for pBIB-KS.

SEQ ID NO: 166 is the determined DNA sequence for the 5' (reverse) primer for pBIB-KS.

SEQ ID NO: 167 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar L2.

SEQ ID NO: 168 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar D.

SEQ ID NO: 169 is the determined full-length DNA sequence for the *C. trachomatis* pmpI gene.

SEQ ID NO: 170 is the determined full-length DNA sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 171 is the determined full-length DNA sequence for the *C. trachomatis* pmpE gene.

SEQ ID NO: 172 is the determined full-length DNA sequence for the C. trachomatis pmpD gene.

SEQ ID NO: 173 is the determined full-length DNA sequence for the C. trachomatis pmpC gene.

SEQ ID NO: 174 is the determined full-length DNA sequence for the C. trachomatis pmpB gene.

SEQ ID NO: 175 is the predicted full-length amino acid sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 176 is the predicted full-length amino acid sequence for the C. $trachomatis\ pmpG$ gene.

SEQ ID NO: 177 is the predicted full-length amino acid sequence for the C. $trachomatis\ pmpE\ gene.$

SEQ ID NO: 178 is the predicted full-length amino acid sequence for the C. $trachomatis\ pmpD$ gene.

SEQ ID NO: 179 is the predicted full-length amino acid sequence for the C. $trachomatis\ pmpC$ gene.

SEQ ID NO: 180 is the predicted full-length amino acid sequence for the *C. trachomatis* pmpB gene.

SEQ ID NO: 181 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 182 is a subsequently determined full-length DNA sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 183 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 184 is a first determined DNA sequence representing the carboxy terminus for the *C. trachomatis* pmpD gene.

SEQ ID NO: 185 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the C. trachomatis pmpD gene.

SEQ ID NO: 186 is a first determined DNA sequence representing the carboxy terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 187 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the C. trachomatis pmpC gene.

SEQ ID NO: 188 is the determined DNA sequence representing the C. pneumoniae serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 189 is the predicted amino acid sequence minus the signal sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 190 is subsequently predicted amino acid sequence for the C. $trachomatis\ pmpG$ gene.

SEQ ID NO: 191 is the predicted amino acid sequence minus the signal sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 192 is a first predicted amino acid sequence representing the carboxy terminus for the *C. trachomatis* pmpD gene.

SEQ ID NO: 193 is a second predicted amino acid sequence representing the Amino terminus minus the signal sequence for the *C. trachomatis* pmpD gene.

SEQ ID NO: 194 is a first predicted amino acid sequence representing the Carboxy terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 195 is a second predicted amino acid sequence representing the Amino terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 196 is the predicted amino acid sequence representing the *C. pneumoniae* serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 197 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

SEQ ID NO: 198 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

SEQ ID NO: 199 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpC gene in the SKB vaccine vector.

SEQ ID NO: 200 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpD gene in the SKB vaccine vector.

SEQ ID NO: 201 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.

SEQ ID NO: 202 is the determined DNA sequence for the insertion sequence for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.

SEQ ID NO: 203 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpE gene in the SKB vaccine vector.

SEQ ID NO: 204 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpE gene in the SKB vaccine vector.

SEQ ID NO: 205 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.

SEQ ID NO: 206 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpG gene in the SKB vaccine vector.

SEQ ID NO: 207 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 208 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the C. trachomatis pmpC gene in the pET17b vector.

SEQ ID NO: 209 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 210 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 211 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 212 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 213 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 214 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 215 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 216 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 217 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

SEQ ID NO: 218 is the amino acid sequence for the insertion sequence for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 219 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpG gene in the pET17b vector.

SEQ ID NO: 220 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpG gene in the pET17b vector.

SEQ ID NO: 221 is the amino acid sequence for the insertion sequence for cloning the C. trachomatis pmpG gene in the pET17b vector.

SEQ ID NO: 222 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpI gene in the pET17b vector.

SEQ ID NO: 223 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpI gene in the pET17b vector.

SEQ ID NO: 224 is the determined amino acid sequence for the C. pneumoniae Swib peptide 1-20.

SEQ ID NO: 225 is the determined amino acid sequence for the C. pneumoniae Swib peptide 6-25.

SEQ ID NO: 226 is the determined amino acid sequence for the C. pneumoniae Swib peptide 12-31.

SEQ ID NO: 227 is the determined amino acid sequence for the C. pneumoniae Swib peptide 17-36.

SEQ ID NO: 228 is the determined amino acid sequence for the C. pneumoniae Swib peptide 22-41.

SEQ ID NO: 229 is the determined amino acid sequence for the $\it C$. pneumoniae Swib peptide 27-46.

SEQ ID NO: 230 is the determined amino acid sequence for the C. pneumoniae Swib peptide 42-61.

SEQ ID NO: 231 is the determined amino acid sequence for the C. pneumoniae Swib peptide 46-65.

SEQ ID NO: 232 is the determined amino acid sequence for the C. pneumoniae Swib peptide 51-70.

SEQ ID NO: 233 is the determined amino acid sequence for the C. pneumoniae Swib peptide 56-75.

SEQ ID NO: 234 is the determined amino acid sequence for the C. pneumoniae Swib peptide 61-80.

SEQ ID NO: 235 is the determined amino acid sequence for the C. pneumoniae Swib peptide 66-87.

SEQ ID NO: 236 is the determined amino acid sequence for the $\it C.$ trachomatis OMCB peptide 103-122.

SEQ ID NO: 237 is the determined amino acid sequence for the C. trachomatis OMCB peptide 108-127.

SEQ ID NO: 238 is the determined amino acid sequence for the C. trachomatis OMCB peptide 113-132.

SEQ ID NO: 239 is the determined amino acid sequence for the C. trachomatis OMCB peptide 118-137.

SEQ ID NO: 240 is the determined amino acid sequence for the C. trachomatis OMCB peptide 123-143.

SEQ ID NO: 241 is the determined amino acid sequence for the C. trachomatis OMCB peptide 128-147.

SEQ ID NO: 242 is the determined amino acid sequence for the C. trachomatis OMCB peptide 133-152.

SEQ ID NO: 243 is the determined amino acid sequence for the C. trachomatis OMCB peptide 137-156.

SEQ ID NO: 244 is the determined amino acid sequence for the C. trachomatis OMCB peptide 142-161.

SEQ ID NO: 245 is the determined amino acid sequence for the C. trachomatis OMCB peptide 147-166.

SEQ ID NO: 246 is the determined amino acid sequence for the C. trachomatis OMCB peptide 152-171.

SEQ ID NO: 247 is the determined amino acid sequence for the C. trachomatis OMCB peptide 157-176.

SEQ ID NO: 248 is the determined amino acid sequence for the C. trachomatis OMCB peptide 162-181.

SEQ ID NO: 249 is the determined amino acid sequence for the C. trachomatis OMCB peptide 167-186.

SEQ ID NO: 250 is the determined amino acid sequence for the C. trachomatis OMCB peptide 171-190.

SEQ ID NO: 251 is the determined amino acid sequence for the C. trachomatis OMCB peptide 171-186.

SEQ ID NO: 252 is the determined amino acid sequence for the C. trachomatis OMCB peptide 175-186.

SEQ ID NO: 252 is the determined amino acid sequence for the C. trachomatis OMCB peptide 175-186.

SEQ ID NO: 253 is the determined amino acid sequence for the C. pneumoniae OMCB peptide 185-198.

SEQ ID NO: 254 is the determined amino acid sequence for the C. trachomatis TSA peptide 96-115.

SEQ ID NO: 255 is the determined amino acid sequence for the C. trachomatis TSA peptide 101-120.

SEQ ID NO: 256 is the determined amino acid sequence for the C. trachomatis TSA peptide 106-125.

SEQ ID NO: 257 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 111-130.

SEQ ID NO: 258 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 116-135.

SEQ ID NO: 259 is the determined amino acid sequence for the C. trachomatis TSA peptide 121-140.

SEQ ID NO: 260 is the determined amino acid sequence for the C. trachomatis TSA peptide 126-145.

SEQ ID NO: 261 is the determined amino acid sequence for the C. trachomatis TSA peptide 131-150.

SEQ ID NO: 262 is the determined amino acid sequence for the C. trachomatis TSA peptide 136-155.

SEQ ID NO: 263 is the determined full-length DNA sequence for the C. trachomatis CT529/Cap 1 gene serovar I.

SEQ ID NO: 264 is the predicted full-length amino sequence for the C. trachomatis CT529/Cap 1 gene serovar I.

SEQ ID NO: 265 is the determined full-length DNA sequence for the C. trachomatis CT529/Cap 1 gene serovar K.

SEQ ID NO: 266 is the predicted full-length amino sequence for the C. trachomatis CT529/Cap 1 gene serovar K.

SEQ ID NO: 267 is the determined DNA sequence for the *C. trachomatis* clone 17-G4-36 sharing homology to part of the ORF of DNA-dirrected RNA polymerase beta subunit- CT315 in serD.

SEQ ID NO: 268 is the determined DNA sequence for the partial sequence of the *C. trachomatis* CT016 gene in clone 2E10.

SEQ ID NO: 269 is the determined DNA sequence for the partial sequence of the C. trachomatis tRNA syntase gene in clone 2E10.

SEQ ID NO: 270 is the determined DNA sequence for the partial sequence for the C. trachomatis clpX gene in clone 2E10.

SEQ ID NO: 271 is a first determined DNA sequence for the *C. trachomatis* clone CtL2gam-30 representing the 5'end.

SEQ ID NO: 272 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-30 representing the 3'end.

SEQ ID NO: 273 is the determined DNA sequence for the C. trachomatis clone CtL2gam-28.

SEQ ID NO: 274 is the determined DNA sequence for the C. trachomatis clone CtL2gam-27.

SEQ ID NO: 275 is the determined DNA sequence for the C. trachomatis clone CtL2gam-26.

SEQ ID NO: 276 is the determined DNA sequence for the C. trachomatis clone CtL2gam-24.

SEQ ID NO: 277 is the determined DNA sequence for the C. trachomatis clone CtL2gam-23.

SEQ ID NO: 278 is the determined DNA sequence for the C. trachomatis clone CtL2gam-21.

SEQ ID NO: 279 is the determined DNA sequence for the C. trachomatis

clone CtL2gam-18.

SEQ ID NO: 280 is the determined DNA sequence for the C. trachomatis clone CtL2gam-17.

SEQ ID NO: 281 is a first determined DNA sequence for the *C. trachomatis* clone CtL2gam-15 representing the 5' end.

SEQ ID NO: 282 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-15 representing the 3' end.

SEQ ID NO: 283 is the determined DNA sequence for the $\it C.$ trachomatis clone CtL2gam-13.

SEQ ID NO: 284 is the determined DNA sequence for the $\it C.$ trachomatis clone CtL2gam-10.

SEQ ID NO: 285 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-8.

SEQ ID NO: 286 is a first determined DNA sequence for the *C. trachomatis* clone CtL2gam-6 representing the 5' end.

SEQ ID NO: 287 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-6 representing the 3' end.

SEQ ID NO: 288 is the determined DNA sequence for the C. trachomatis clone CtL2gam-5.

SEQ ID NO: 289 is the determined DNA sequence for the C. trachomatis clone CtL2gam-2.

SEQ ID NO: 290 is the determined DNA sequence for the $\it C.$ trachomatis clone CtL2gam-1.

SEQ ID NO: 291 is the determined full-length DNA sequence for the C. pneumoniae homologue of the CT529 gene.

SEQ ID NO: 292 is the predicted full-length amino acid sequence for the C. pneumoniae homologue of the CT529 gene.

SEQ ID NO: 293 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpG gene in the SKB vaccine vector.

DESCRIPTION OF THE FIGURES

Fig. 1 illustrates induction of INF-γ from a *Chlamydia*-specific T cell line activated by target cells expressing clone 4C9-18#2.

Fig. 2 illustrates retroviral vectors pBIB-KS1,2,3 modified to contain a Kosak translation initiation site and stop codons.

Fig. 3 shows specific lysis in a chromium release assay of P815 cells pulsed with *Chlamydia* peptides CtC7.8-12 (SEQ ID NO: 18) and CtC7.8-13 (SEQ ID NO: 19).

Fig. 4 shows antibody isotype titers in C57Bl/6 mice immunized with C. trachomatis SWIB protein.

Fig. 5 shows *Chlamydia*-specific T-cell proliferative responses in splenocytes from C3H mice immunized with *C. trachomatis* SWIB protein.

Fig. 6 illustrates the 5' and 3' primer sequences designed from *C. pneumoniae* which were used to isolate the SWIB and S13 genes from *C. pneumoniae*.

Figs. 7A and 7B show induction of IFN-γ from a human anti-chlamydia T-cell line (TCL-8) capable of cross-reacting to *C. trachomatis* and *C. pneumonia* upon activation by monocyte-derived dendritic cells expressing chlamydial proteins.

Fig. 8 shows the identification of T cell epitopes in Chlamydial ribosomal S13 protein with T-cell line TCL 8 EB/DC.

Fig. 9 illustrates the proliferative response of CP-21 T-cells generated against *C. pnuemoniae*-infected dendritic cells to recombinant *C. pneumonia*-SWIBprotein, but not *C. trachomatis* SWIB protein.

Fig. 10 shows the *C. trachomatis*-specific SWIB proliferative responses of a primary T-cell line (TCT-10 EB) from an asymptomatic donor.

Fig. 11 illustrates the identification of T-cell epitope in *C. trachomatis* SWIB with an antigen specific T-cell line (TCL-10 EB).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the diagnosis and treatment of Chlamydial infection. In one aspect, the compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a *Chlamydia* antigen, or a variant thereof.

In specific embodiments, the subject invention discloses polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, wherein the *Chlamydia* antigen comprises an amino acid sequence encoded by a polynucleotide molecule including a sequence selected from the group consisting of (a) nucleotide sequences recited in SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290 (b) the complements of said nucleotide sequences, and (c) variants of such sequences.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e., antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the inventive antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *Chlamydia* antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

An "immunogenic portion" of an antigen is a portion that is capable of reacting with sera obtained from a *Chlamydia*-infected individual (*i.e.*, generates an absorbance reading with sera from infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). Such immunogenic portions generally comprise at least about 5 amino acid residues, more preferably at least about 10, and most

preferably at least about 20 amino acid residues. Methods for preparing and identifying immunogenic portions of antigens of known sequence are well known in the art and include those summarized in Paul, Fundamental Immunology, 3rd ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native Chlamydia protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, 125 I-labeled Protein A.

Examples of immunogenic portions of antigens contemplated by the present invention include, for example, the T cell stimulating epitopes provided in SEQ ID NO: 9, 10, 18, 19, 31, 39, 93-96, 98, 100-102, 106, 108, 138-140, 158, 167, 168, 246, 247 and 254-256. Polypeptides comprising at least an immunogenic portion of one or more *Chlamydia* antigens as described herein may generally be used, alone or in combination, to detect Chlamydial infection in a patient.

The compositions and methods of the present invention also encompass variants of the above polypeptides and polynucleotide molecules. Such variants include, but are not limited to, naturally occurring allelic variants of the inventive sequences. In particular, variants include other *Chlamydiae* serovars, such as serovars D, E and F, as well as the several LGV serovars which share homology to the inventive polypeptide and

polynucleotide molecules described herein. Preferably, the serovar homologues show 95-99% homology to the corresponding polypeptide sequence(s) described herein.

A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such that the antigenic properties of the polypeptide are retained. In a preferred embodiment, variant polypeptides differ from an identified sequence by substitution, deletion or addition of five amino acids or fewer. Such variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the antigenic properties of the modified polypeptide using, for example, the representative procedures described herein. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (e.g., 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein. Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity (determined as described below) to the identified polypeptides.

As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent

conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide. Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A polynucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions such that the immunogenicity of the encoded polypeptide is not diminished, relative to the native protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis as taught, for example, by Adelman et al. (DNA, 2:183, 1983). Nucleotide variants may be naturally occurring allelic variants as discussed below, or non-naturally occurring variants. Variant nucleotide sequences preferably exhibit at least about 70%, more preferably at least about 80% and most preferably at least about 90% identity (determined as described below) to the recited sequence.

The polypeptides provided by the present invention include variants that are encoded by polynucleotide sequences which are substantially homologous to one or more of the polynucleotide sequences specifically recited herein. "Substantial homology," as used herein, refers to polynucleotide sequences that are capable of hybridizing under moderately

stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species homology, at 45°C with 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Such hybridizing polynucleotide sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode a polypeptide that is the same as a polypeptide of the present invention.

Two nucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Resarch Foundaiton, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) Fast and sensitive multiple sequence alignments on a microcomputer *CABIOS* 5:151-153; Myers, E.W. and Muller W. (1988) Optimal alignments in linear space *CABIOS* 4:11-17; Robinson, E.D. (1971) *Comb. Theor 11*:105; Santou, N. Nes, M. (1987) The neighbor joining method. A new method for reconstructing phylogenetic trees *Mol. Biol. Evol.* 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy* – the Principles and Practice of Numerical Taxonomy, Freeman Press, San

Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) Rapid similarity searches of nucleic acid and protein data banks *Proc. Natl. Acad., Sci. USA* 80:726-730.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Also included in the scope of the present invention are alleles of the genes encoding the nucleotide sequences recited in herein. As used herein, an "allele" or "allellic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone or in combination with the others, one or more times in a given sequence. In specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a Chlamydia antigen (or a variant of such an antigen), that comprises one or more of the amino acid sequences encoded by (a) a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1-4, 15 21-25, 44-64, 66-76 and 79-88; (b) the complements of such DNA sequences or (c) DNA sequences substantially homologous to a sequence in (a) or (b). As discussed in the Examples below, several of the Chlamydia antigens disclosed herein recognize a T cell line that recognizes both Chlamydia trachomatis and Chlamydia pneumoniae infected monocyte-derived dendritic cells, indicating that they may represent an immunoreactive epitope shared by Chlamydia trachomatis and

Chlamydia pneumoniae. The antigens may thus be employed in a vaccine for both C. trachomatis genital tract infections and for C. pneumonia infections. Further characterization of these Chlamydia antigens from Chlamydia trachomatis and Chlamydia pneumonia to determine the extent of cross-reactivity is provided in Example 6. Additionally, Example 4 describes cDNA fragments (SEQ ID NO: 15, 16 and 33) isolated from C. trachomatis which encode proteins (SEQ ID NO: 17-19 and 32) capable of stimulating a Chlamydia-specific murine CD8+ T cell line.

In general, Chlamydia antigens, and polynucleotide sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, polynucleotide molecules encoding Chlamydia antigens may be isolated from a Chlamydia genomic or cDNA expression library by screening with a Chlamydia-specific T cell line as described below, and sequenced using techniques well known to those of skill in the art. Additionally, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for Chlamydia-associated expression (i.e., expression that is at least two fold greater in Chlamydia-infected cells than in controls, as determined using a representative assay provided herein). Such screens may be performed using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., Proc. Natl. Acad. Sci. USA 93:10614-10619, 1996 and Heller et al., Proc. Natl. Acad. Sci. USA 94:2150-2155, 1997). Alternatively, polypeptides may be amplified from cDNA prepared from cells expressing the proteins described herein. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

Antigens may be produced recombinantly, as described below, by inserting a polynucleotide sequence that encodes the antigen into an expression vector and expressing the antigen in an appropriate host. Antigens may be evaluated for a desired property, such as the ability to react with sera obtained from a *Chlamydia*-infected individual as described herein, and may be sequenced using, for example, traditional Edman chemistry. *See* Edman and Berg, *Eur. J. Biochem.* 80:116-132, 1967.

Polynucleotide sequences encoding antigens may also be obtained by screening an appropriate *Chlamydia* cDNA or genomic DNA library for polynucleotide sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated antigens. Degenerate oligonucleotide sequences for use in such a screen may be designed and synthesized, and the screen may be performed, as described (for example) in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using the above oligonucleotides in methods well known in the art, to isolate a nucleic acid probe from a cDNA or genomic library. The library screen may then be performed using the isolated probe.

An amplified portion may be used to isolate a full length gene from a suitable library (e.g., a Chlamydia cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ³²P) using well known techniques. A bacterial or bacteriophage library is then screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences are then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known

techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using techniques well known in the art (see, for example, Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263, 1987; Erlich ed., PCR Technology, Stockton Press, NY, 1989), and software well known in the art may also be employed. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (see Triglia et al., Nucl. Acids Res. 16:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Additional techniques include capture PCR (Lagerstrom et al., PCR Methods Applic. 1:111-19, 1991) and walking PCR (Parker et al., Nucl. Acids. Res. 19:3055-60, 1991). Transcription-Mediated Amplification, or TMA is another method that may be utilized for the amplification of DNA, rRNA, or mRNA, as described in Patent No. PCT/US91/03184. This autocatalytic and isothermic non-PCR based method utilizes two primers and two enzymes: RNA polymerase and reverse transcriptase. One primer contains a promoter sequence for RNA polymerase. In the first amplification, the promoter-primer hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the 3'end of the promoter-primer. The RNA in the resulting complex is degraded and a second primer binds to the DNA copy. A

new strand of DNA is synthesized from the end of the primer by reverse transcriptase creating double stranded DNA. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA amplicons re-enters the TMA process and serves as a template for a new round of replication leading to the expotential expansion of the RNA amplicon. Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length cDNA sequences may also be obtained by analysis of genomic fragments.

Polynucleotide variants may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (see Adelman et al., DNA 2:183, 1983). Alternatively, RNA molecules may be generated by in vitro or in vivo transcription of DNA sequences encoding a Chlamydial protein, or portion thereof, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated in vivo (e.g., by transfecting antigen-presenting cells, such as dendritic cells, with a cDNA construct encoding a Chlamydial polypeptide, and administering the transfected cells to the patient).

A portion of a sequence complementary to a coding sequence (i.e., an antisense polynucleotide) may also be used as a probe or to modulate gene expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells of tissues to facilitate the production of antisense RNA. An antisense polynucleotide may be used, as described herein, to inhibit expression of a *Chlamydial* protein. Antisense technology can be used to control gene expression through triple-helix formation, which

compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (see Gee et al., In Huber and Carr, Molecular and Immunologic Approaches, Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (e.g., promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

A portion of a coding sequence, or of a complementary sequence, may also be designed as a probe or primer to detect gene expression. Probes may be labeled with a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers, as noted above, are preferably 22-30 nucleotides in length.

Any polynucleotide may be further modified to increase stability in vivo. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothicate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where

amino acids are sequentially added to a growing amino acid chain. See Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division, Foster City, CA, and may be operated according to the manufacturer's instructions.

As noted above, immunogenic portions of *Chlamydia* antigens may be prepared and identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3d ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptide portions of the native antigen for immunogenic properties. The representative ELISAs described herein may generally be employed in these screens. An immunogenic portion of a polypeptide is a portion that, within such representative assays, generates a signal in such assays that is substantially similar to that generated by the full length antigen. In other words, an immunogenic portion of a *Chlamydia* antigen generates at least about 20%, and preferably about 100%, of the signal induced by the full length antigen in a model ELISA as described herein.

Portions and other variants of *Chlamydia* antigens may be generated by synthetic or recombinant means. Variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Sections of the polynucleotide sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

Recombinant polypeptides containing portions and/or variants of a native antigen may be readily prepared from a polynucleotide sequence encoding the polypeptide using a variety of techniques well known to those of ordinary skill in the art. For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides as described herein. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an

expression vector containing a polynucleotide molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line, such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in an isolated, substantially pure, form. Preferably, the polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known Chlamydial protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native Certain preferred fusion partners are both immunological and recombinant protein. expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein. A DNA sequence encoding a fusion protein of the present invention may be constructed using known recombinant DNA techniques to assemble separate DNA sequences encoding, for example, the first and second polypeptides, into an appropriate expression vector. The 3' end of a DNA sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the

fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene 40*:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA 83*:8258-8562, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. As an alternative to the use of a peptide linker sequence (when desired), one can utilize non-essential N-terminal amino acid regions (when present) on the first and second polypeptides to separate the functional domains and prevent steric hindrance.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided that comprise a polypeptide of the present invention together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (see, for example, Stoute et al. New Engl. J. Med., 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (e.g., the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in E. coli (thus functioning as

an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene 43*:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology 10*:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305. Additionally, the fusion protein Ra12 may be linked to the inventive polynucleotides to facilitate protein expression.

In another aspect, the present invention provides methods for using one or more of the above polypeptides or fusion proteins (or polynucleotides encoding such polypeptides or fusion proteins) to induce protective immunity against Chlamydial infection in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat Chlamydial infection.

In this aspect, the polypeptide, fusion protein or polynucleotide molecule is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines may comprise one or more of the above polypeptides and an immunostimulant,

such as an adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other *Chlamydia* antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

Alternatively, a vaccine may contain polynucleotides encoding one or more polypeptides or fusion proteins as described above, such that the polypeptide is generated in situ. In such vaccines, the polynucleotides may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary polynucleotide sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the polynucleotides may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective) virus. Techniques for incorporating polynucleotides into such expression systems are well known to those of ordinary skill in the art. The polynucleotides may also be administered as "naked" plasmid vectors as described, for example, in Ulmer et al., Science 259:1745-1749, 1993 and reviewed by Cohen, Science 259:1691-1692, 1993. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The uptake of naked polynucleotides may be increased by incorporating the polynucleotides into and/or onto biodegradable beads, which are efficiently transported into the cells. The preparation and use of such systems is well known in the art.

In a related aspect, a polynucleotide vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known *Chlamydia* antigen. For example, administration of polynucleotides encoding a polypeptide of the present invention, either "naked" or in a delivery system as described above, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Polypeptides and polynucleotides disclosed herein may also be employed in adoptive immunotherapy for the treatment of *Chlamydial* infection. Adoptive immunotherapy may be broadly classified into either active or passive immunotherapy. In active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system with the administration of immune response-modifying agents (for example, vaccines, bacterial adjuvants, and/or cytokines).

In passive immunotherapy, treatment involves the delivery of biologic reagents with established immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate anti-*Chlamydia* effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocyte, CD4+ T-helper), killer cells (such as Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-idiotypic antibodies (as in U.S. Patent No. 4,918,164), for passive immunotherapy.

The predominant method of procuring adequate numbers of T-cells for adoptive immunotherapy is to grow immune T-cells *in vitro*. Culture conditions for expanding single antigen-specific T-cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. These *in vitro* culture conditions typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast, or B-cells, may be pulsed with

immunoreactive polypeptides, or polynucleotide sequence(s) may be introduced into antigen presenting cells, using a variety of standard techniques well known in the art. For example, antigen presenting cells may be transfected or transduced with a polynucleotide sequence, wherein said sequence contains a promoter region appropriate for increasing expression, and can be expressed as part of a recombinant virus or other expression system. Several viral vectors may be used to transduce an antigen presenting cell, including pox virus, vaccinia virus, and adenovirus; also, antigen presenting cells may be transfected with polynucleotide sequences disclosed herein by a variety of means, including gene-gun technology, lipidmediated delivery, electroporation, osmotic shock, and particlate delivery mechanisms, resulting in efficient and acceptable expression levels as determined by one of ordinary skill in the art. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term in vivo. Studies have demonstrated that cultured T-cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever, M., et al, "Therapy With Cultured T Cells: Principles Revisited," Immunological Reviews, 157:177, 1997).

The polypeptides disclosed herein may also be employed to generate and/or isolate chlamydial-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by *in vivo* immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ or CD4+ T-cell clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

Alternatively, peptides corresponding to immunogenic portions of the polypeptides may be employed to generate *Chlamydia* reactive T cell subsets by selective *in vitro* stimulation and expansion of autologous T cells to provide antigen-specific T cells which may be subsequently transferred to the patient as described, for example, by Chang *et al, (Crit. Rev. Oncol. Hematol., 22*(3), 213, 1996). Cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as IsolexTM System, available from Nexell Therapeutics, Inc. Irvine, CA. The separated cells are stimulated with one or more of the immunoreactive

polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T cells. The population of antigen-specific T cells is then expanded using standard techniques and the cells are administered back to the patient.

In other embodiments, T-cell and/or antibody receptors specific for the polypeptides disclosed herein can be cloned, expanded, and transferred into other vectors or effector cells for use in adoptive immunotherapy. In particular, T cells may be transfected with the appropriate genes to express the variable domains from chlamydia specific monoclonal antibodies as the extracellular recognition elements and joined to the T cell receptor signaling chains, resulting in T cell activation, specific lysis, and cytokine release. This enables the T cell to redirect its specificity in an MHC-independent manner. See for example, Eshhar, Z., Cancer Immunol Immunother, 45(3-4):131-6, 1997 and Hwu, P., et al, Cancer Res, 55(15):3369-73, 1995. Another embodiment may include the transfection of chlamydia antigen specific alpha and beta T cell receptor chains into alternate T cells, as in Cole, DJ, et al, Cancer Res, 55(4):748-52, 1995.

In a further embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides corresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-specific dendritic cells may either be transferred into a patient, or employed to stimulate T cells to provide antigen-specific T cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T cells and the subsequent use of such antigen-specific T cells to eradicate disease in a murine model has been demonstrated by Cheever et al, *Immunological Reviews*, 157:177, 1997). Additionally, vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated *in vitro* for autologous transplant back into the same patient.

Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents disclosed herein may be incorporated into pharmaceutical compositions or immunogenic compositions (i.e., vaccines). Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds and an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response to an exogenous antigen.

Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g., polylactic galactide) and liposomes (into which the compound is incorporated; see e.g., Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other *Chlamydial* antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated in situ. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, Crit. Rev. Therap. Drug Carrier Systems 15:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch et al., Proc. Natl. Acad. Sci. USA 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, Biotechniques 6:616-627, 1988; Rosenfeld et al., Science 252:431-434, 1991; Kolls et al., Proc. Natl. Acad. Sci. USA 91:215-219, 1994; Kass-Eisler et al., Proc. Natl. Acad. Sci. USA 90:11498-11502, 1993; Guzman et al., Circulation 88:2838-2848, 1993; and Guzman et al., Cir. Res. 73:1202-1207, 1993.

Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide) and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium tuberculosis derived proteins. Suitable adjuvants are commercially available

as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, under select circumstances, the adjuvant composition may be designed to induce an immune response predominantly of the Th1 type or Th2 type. High levels of Th1-type cytokines (e.g., IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, Ann. Rev. Immunol. 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Ribi ImmunoChem Research Inc. (Hamilton, MT) (see US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555. Another preferred adjuvant is a saponin, preferably QS21, which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations

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comprises an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210. Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient.

The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets *Chlamydia*-infected cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-*Chlamydia* effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs

(Banchereau and Steinman, Nature 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic immunity (see Timmerman and Levy, Ann. Rev. Med. 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate in situ, with marked cytoplasmic processes (dendrites) visible in vitro), their ability to take up, process and present antigens with high efficiency, and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells in vivo or ex vivo, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (see Zitvogel et al., Nature Med. 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcy receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a

Chlamydial protein (or portion or other variant thereof) such that the Chlamydial polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place ex vivo, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs in vivo. In vivo and ex vivo transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., Immunology and cell Biology 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the Chlamydial polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Routes and frequency of administration of pharmaceutical compositions and vaccines, as well as dosage, will vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (e.g., intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from *Chlamydial* infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 µg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a *Chlamydial* protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

In another aspect, the present invention provides methods for using the polypeptides described above to diagnose Chlamydial infection. In this aspect, methods are provided for detecting Chlamydial infection in a biological sample, using one or more of the above polypeptides, either alone or in combination. For clarity, the term "polypeptide" will be used when describing specific embodiments of the inventive diagnostic methods. However, it will be clear to one of skill in the art that the fusion proteins of the present invention may also be employed in such methods.

As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient. The polypeptides are used in an assay, as described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative

to a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to *Chlamydia* antigens which may be indicative of *Chlamydia*-infection.

In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (i.e., one component polypeptide will tend to detect infection in samples where the infection would not be detected by another component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with *Chlamydia*. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested.

A variety of assay formats are known to those of ordinary skill in the art for using one or more polypeptides to detect antibodies in a sample. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988, which is incorporated herein by reference. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (e.g., in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate, or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The polypeptides may be bound to the solid support using a variety of techniques known to those of ordinary skill in the art. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 µg, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin (BSA) or Tween 20™ (Sigma Chemical Co., St. Louis, MO) may be employed. The

immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable dilutent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (i.e., incubation time) is that period of time that is sufficient to detect the presence of antibody within an HGE-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20TM. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (e.g., Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods

are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-Chlamydia antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for Chlamydia-infection. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., Clinical Epidemiology: A Basic Science for Clinical Medicine, Little Brown and Co., 1985, pp. 106-107. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (i.e., the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for Chlamydial infection.

In a related embodiment, the assay is performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (e.g., protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be

performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-Chlamydia antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 µg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (e.g., one drop) of patient serum or blood.

Of course, numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only. One example of an alternative assay protocol which may be usefully employed in such methods is a Western blot, wherein the proteins present in a biological sample are separated on a gel, prior to exposure to a binding agent. Such techniques are well known to those of skill in the art.

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a *Chlamydial* protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a *Chlamydial* protein if it reacts at a detectable level (within, for example, an ELISA) with a *Chlamydial* protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10³

L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a *Chlamydial* infection using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a *Chlamydial* protein will generate a signal indicating the presence of a *Chlamydial* infection in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without infection. To determine whether a binding agent satisfies this requirement, biological samples (e.g., blood, sera, sputum urine and/or tissue biopsies) from patients with and without *Chlamydial* infection (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and

the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

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Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A*

Laboratory Manual, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ⁹⁰Y, ¹²³I, ¹²⁵I, ¹³¹I, ¹⁸⁶Re, ¹⁸⁸Re, ²¹¹At, and ²¹²Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, e.g., U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion

of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (e.g., U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (e.g., U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (e.g., U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (e.g., U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (e.g., U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (e.g., U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (e.g., U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (e.g., U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or

in site-specific regions by appropriate methods. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density, and the rate of clearance of the antibody.

Antibodies may be used in diagnostic tests to detect the presence of *Chlamydia* antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting Chlamydial infection in a patient.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify *Chlamydia*-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

As used herein, the term "oligonucleotide primer/probe specific for a DNA molecule" means an oligonucleotide sequence that has at least about 80%, preferably at least about 90% and more preferably at least about 95%, identity to the DNA molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (see, for example, Mullis et al. Ibid; Ehrlich, Ibid). Primers or probes may thus be used to detect Chlamydia-specific sequences in biological samples. DNA probes or primers

comprising oligonucleotide sequences described above may be used alone or in combination with each other.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

ISOLATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS

Chlamydia antigens of the present invention were isolated by expression cloning of a genomic DNA library of Chlamydia trachomatis LGV II essentially as described by Sanderson et al. (J. Exp. Med., 1995, 182:1751-1757) and were shown to induce PBMC proliferation and IFN-γ in an immunoreactive T cell line.

A Chlamydia-specific T cell line was generated by stimulating PBMCs from a normal donor with no history of chlamydial genital tract infection with elementary bodies of Chlamydia trachomatis LGV II. This T cell line, referred to as TCL-8, was found to recognize both Chlamydia trachomatis and Chlamydia pneumonia infected monocyte-derived dendritic cells.

A randomly sheared genomic library of *Chlamydia trachomatis* LGV II was constructed in Lambda ZAP (Stratagene, La Jolla, CA) and the amplified library plated out in 96 well microtiter plates at a density of 30 clones/well. Bacteria were induced to express recombinant protein in the presence of 2 mM IPTG for 3 h, then pelleted and resuspended in 200 μl of RPMI 10% FBS. 10 μl of the induced bacterial suspension was transferred to 96 well plates containing autologous monocyte-derived dendritic cells. After a 2 h incubation, dendritic cells were washed to remove free *E. coli* and *Chlamydia*-specific T cells were added. Positive *E. coli* pools were identified by determining IFN-γ production and proliferation of the T cells in response to the pools.

Four positive pools were identified, which were broken down to yield four pure clones (referred to as 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31), with insert sizes of 481 bp, 183 bp, 110 bp and 1400 bp, respectively. The determined DNA sequences for 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31 are provided in SEQ ID NO: 1-4, respectively. Clone 1-B1-66 is approximately in region 536690 of the *C. trachomatis* genome (NCBI *C.*

trachomatis database). Within clone 1-B1-66, an open reading frame (ORF) has been identified (nucleotides 115 - 375) that encodes a previously identified 9 kDa protein (Stephens, et al. Genbank Accession No. AE001320), the sequence of which is provided in SEQ ID NO: 5). Clone 4-D7-28 is a smaller region of the same ORF (amino acids 22-82 of 1-B1-66). Clone 3-G3-10 is approximately in region 74559 of the *C. trachomatis* genome. The insert is cloned in the antisense orientation with respect to its orientation in the genome. The clone 10-C10-31 contains an open reading frame that corresponds to a previously published sequence for S13 ribosomal protein from *Chlamydia trachomatis* (Gu, L. et al. *J. Bacteriology*, 177:2594-2601, 1995). The predicted protein sequences for 4-D7-28 and 10-C10-31 are provided in SEQ ID NO: 6 and 12, respectively. Predicted protein sequences for 3-G3-10 are provided in SEQ ID NO: 7-11.

In a related series of screening studies, an additional T cell line was used to screen the genomic DNA library of *Chlamydia trachomatis* LGV II described above. A *Chlamydia*-specific T cell line (TCT-1) was derived from a patient with a chlamydial genital tract infection by stimulating patient PBMC with autologous monocyte-derived dendritic cells infected with elementary bodies of *Chlamydia trachomatis* LGV II. One clone, 4C9-18 (SEQ ID NO: 21), containing a 1256 bp insert, elicited a specific immune response, as measured by standard proliferation assays, from the *Chlamydia*-specific T cell line TCT-1. Subsequent analysis revealed this clone to contain three known sequences: lipoamide dehydrogenase (Genbank Accession No. AE001326), disclosed in SEQ ID NO: 22; a hypothetical protein CT429 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 23; and part of an open reading frame of ubiquinone methyltransferase CT428 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 24.

In further studies involving clone 4C9-18 (SEQ ID NO: 21), the full-length amino acid sequence for lipoamide dehydrognase (SEQ ID NO: 22) from *C. trachomatis* (LGV II) was expressed in clone CtL2-LPDA-FL, as disclosed in SEQ ID NO: 90.

To further characterize the open reading frame containing the T cell stimulating epitope(s), a cDNA fragment containing nucleotides 1-695 of clone 4C9-18 with a cDNA sequence encoding a 6X-Histidine tag on the amino terminus was subcloned into the NdeI/EcoRI site of the pET17b vector (Novagen, Madison, WI), referred to as clone 4C9-

18#2 BL21 pLysS (SEQ ID NO: 25, with the corresponding amino acid sequence provided in SEQ ID NO: 26) and transformed into *E. coli*. Selective induction of the transformed *E. coli* with 2 mM IPTG for three hours resulted in the expression of a 26 kDa protein from clone 4C9-18#2 BL21 pLysS, as evidenced by standard Coomassie-stained SDS-PAGE. To determine the immunogenicity of the protein encoded by clone 4C9-18#2 BL21 pLysS, *E. coli* expressing the 26 kDa protein were titered onto 1 x 10⁴ monocyte-derived dendritic cells and incubated for two hours. The dendritic cell cultures were washed and 2.5 x 10⁴ T cells (TCT-1) added and allowed to incubate for an additional 72 hours, at which time the level of IFN-γ in the culture supernatant was determined by ELISA. As shown in Fig. 1, the T-cell line TCT-1 was found to respond to induced cultures as measured by IFN-g, indicating a *Chlamydia*-specific T-cell response against the lipoamide dehydrogenase sequence. Similarly, the protein encoded by clone 4C9-18#2 BL21 pLysS was shown to stimulate the TCT-1 T-cell line by standard proliferation assays.

Subsequent studies to identify additional *Chlamydia trachomatis* antigens using the above-described CD4+ T-cell expression cloning technique yielded additional clones. The TCT-1 and TCL-8 *Chlamydia*-specific T-cell lines, as well as the TCP-21 T-cell line were utilized to screen the *Chlamydia trachomatis* LGVII genomic library. The TCP-21 T-cell line was derived from a patient having a humoral immune response to *Chlamydia pnuemoniae*. The TCT-1 cell line identified 37 positive pools, the TCT-3 cell line identified 41 positive pools and the TCP-21 cell line identified 2 positive pools. The following clones were derived from 10 of these positive pools. Clone 11-A3-93 (SEQ ID NO: 64), identified by the TCP-21 cell line, is a 1339 bp genomic fragment sharing homology to the HAD superfamily (CT103). The second insert in the same clone shares homology with the fab I gene (CT104) present on the complementary strand. Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of *C. pnuemoniae*.

Clone 11-G10-46, (SEQ ID NO: 62), identified using the TCT-3 cell line, contains a 688 bp insert that shares homology to the hypothetical protein CT610. Clone 11-G1-34, (SEQ ID NO: 61), identified using the TCT-3 cell line, has two partial open reading

frames (ORF) with an insert size of 1215 bp. One ORF shares homology to the malate dehydrogenase gene (CT376), and the other ORF shares homology to the glycogen hydrolase gene (CT042). Clone 11-H3-68, (SEQ ID NO: 60), identified using the TCT-3 cell line, has two ORFs with a total insert size of 1180 bp. One partial ORF encodes the plasmid-encoded PGP6-D virulence protein while the second ORF is a complete ORF for the L1 ribosomal gene (CT318). Clone 11-H4-28, (SEQ ID NO: 59), identified using the TCT-3 cell line, has an insert size of 552 bp and is part of the ORF for the dnaK gene (CT396). Clone 12-B3-95, (SEQ ID NO: 58), identified using the TCT-1 cell line, has an insert size of 463 bp and is a part of the ORF for the lipoamide dehydrogenase gene (CT557). Clones 15-G1-89 and 12-B3-95 are identical, (SEQ ID NO: 55 and 58, respectively), identified using the TCT-1 cell line, has an insert size of 463 bp and is part of the ORF for the lipoamide dehydrogenase gene (CT557). Clone 12-G3-83, (SEQ ID NO: 57), identified using the TCT-1 cell line, has an insert size of 1537 bp and has part of the ORF for the hypothetical protein CT622.

Clone 23-G7-68, (SEQ ID NO: 79), identified using the TCT-3 cell line, contains a 950 bp insert and contains a small part of the L11 ribosomal ORF, the entire ORF for L1 ribosomal protein and a part of the ORF for L10 ribosomal protein. Clone 22-F8-91, (SEQ ID NO: 80), identified using the TCT-1 cell line, contains a 395 bp insert that contains a part of the pmpC ORF on the complementary strand of the clone. Clone 21-E8-95, (SEQ ID NO: 81), identified using the TCT-3 cell line, contains a 2,085 bp insert which contains part of CT613 ORF, the complete ORF for CT612, the complete ORF for CT611 and part of the ORF for CT610. Clone 19-F12-57, (SEQ ID NO: 82), identified using the TCT-3 cell line, contains a 405 bp insert which contains part of the CT 858 ORF and a small part of the recA ORF. Clone 19-F12-53, (SEQ ID NO: 83), identified using the TCT-3 cell line, contains a 379 bp insert that is part of the ORF for CT455 encoding glutamyl tRNA synthetase. Clone 19-A5-54, (SEQ ID NO: 84), identified using the TCT-3 cell line, contains a 715 bp insert that is part of the ORF3 (complementary strand of the clone) of the cryptic plasmid. Clone 17-E11-72, (SEQ ID NO: 85), identified using the TCT-1 cell line, contains a 476 bp insert that is part of the ORF for Opp_2 and pmpD. The pmpD region of this clone is covered by the pmpD region of clone 15-H2-76. Clone 17-C1-77, (SEQ ID NO: 86), identified using the TCT-3 cell line, contains a 1551 bp insert that is part of the CT857 ORF, as well as part of

the CT858 ORF. Clone 15-H2-76, (SEQ ID NO: 87), identified using the TCT-1 cell line, contains a 3,031 bp insert that contains a large part of the pmpD ORF, part of the CT089 ORF, as well as part of the ORF for SycE. Clone 15-A3-26, (SEQ ID NO: 88), contains a 976 bp insert that contains part of the ORF for CT858. Clone 17-G4-36, (SEQ ID NO: 267), identified using the TCT-10 cell line, contains a 680 bp insert that is in frame with beta-gal in the plasmid and shares homology to part of the ORF for DNA-directed RNA polymerase beta subunit (CT315 in SerD).

Several of the clones described above share homology to various polymorphic membrane proteins. The genomic sequence of *Chlamydia trachomatis* contains a family of nine polymorphic membrane protein genes, referred to as pmp. These genes are designated pmpA, pmpB, pmpC, pmpD, pmpE, pmpF, pmpG, pmpH and pmpI. Proteins expressed from these genes are believed to be of biological relevance in generating a protective immune response to a *Chlamydial* infection. In particular, pmpC, pmpD, pmpE and pmpI contain predictable signal peptides, suggesting they are outer membrane proteins, and therefore, potential immunological targets.

Based on the *Chlamydia trachomatis* LGVII serovar sequence, primer pairs were designed to PCR amplify the full-length fragments of pmpC, pmpD, pmpE, pmpG, pmpH and pmpI. The resulting fragments were subcloned into the DNA vaccine vector JA4304 or JAL, which is JA4304 with a modified linker (SmithKline Beecham, London, England). Specifically, PmpC was subcloned into the JAL vector using the 5' oligo GAT AGG CGC GCC GCA ATC ATG AAA TTT ATG TCA GCT ACT GCT G and the 3' oligo CAG AAC GCG TTT AGA ATG TCA TAC GAG CAC CGC A, as provided in SEQ ID NO: 197 and 198, respectively. PCR amplification of the gene under conditions well known in the art and ligation into the 5' ASCI/3' MluI sites of the JAL vector was completed after inserting the short nucleotide sequence GCAATC (SEQ ID NO: 199) upstream of the ATG to create a Kozak-like sequence. The resulting expression vector contained the full-length pmpC gene comprising 5325 nucleotides (SEQ ID NO: 173) containing the hypothetical signal sequence, which encodes a 187 kD protein (SEQ ID NO: 179). The pmpD gene was subcloned into the JA4304 vaccine vector following PCR amplification of the gene using the following oligos: 5' oligo- TGC AAT CAT GAG TTC GCA GAA AGA TAT AAA AAG C

(SEQ ID NO: 200) and 3' oligo- CAG AGC TAG CTT AAA AGA TCA ATC GCA ATC CAG TAT TC (SEQ ID NO: 201). The gene was ligated into the a 5' blunted HIII/3' MluI site of the JA4304 vaccine vector using standard techniques well known in the art. The CAATC (SEQ ID NO: 202) was inserted upstream of the ATG to create a Kozak-like sequence. This clone is unique in that the last threonine of the HindIII site is missing due to the blunting procedure, as is the last glycine of the Kozak-like sequence. The insert, a 4593 nucleotide fragment (SEQ ID NO: 172) is the full-length gene for pmpD containing the hypothetical signal sequence, which encodes a 161 kD protein (SEQ ID NO: 178). PmpE was subcloned into the JA4304 vector using the 5' oligo- TGC AAT CAT GAA AAA AGC GTT TTT CTT TTT C (SEQ ID NO: 203), and the 3' oligo- CAG AAC GCG TCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 204). Following PCR amplification, the gene was ligated into the 5' blunted HIII/3' MluI site of JA4304. To facilitate this, a short nucleotide sequence, TGCAATC (SEQ ID NO: 293), was added upstream of the initiation codon for creating a Kozak-like sequence and reconstituting the HindIII site. The insert is the full-length pmpE gene (SEQ ID NO: 171) containing the hypothetical signal sequence. The pmpE gene encodes a 105 kD protein (SEQ ID NO: 177). The pmpG gene was PCR amplified using the 5' oligo- GTG CAA TCA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 205), and the 3' oligo- CAG AAC GCG TTT AGA ACC GGA CTT TAC TTC C (SEQ ID NO: 206) and subcloned into the JA4304 vector. Similar cloning strategies were followed for the pmpI and pmpK genes. In addition, primer pairs were designed to PCR amplify the full-length or overlapping fragments of the pmp genes, which were then subcloned for protein expression in the pET17b vector (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Several of the genes encoding the recombinant proteins, as described below, lack the native signal sequence to facilitate expression of the protein. Full-length protein expression of pmpC was accomplished through expression of two overlapping fragments, representing the amino and carboxy termini. Subcloning of the pmpC-amino terminal portion, which lacks the signal sequence, (SEQ ID NO: 187, with the corresponding amino acid sequence provided in SEQ ID NO: 195) used the 5' oligo- CAG ACA TAT GCA TCA CCA TCA CCA TCA CGA

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GGC GAG CTC GAT CCA AGA TC (SEQ ID NO: 207), and the 3' oligo- CAG AGG TAC CTC AGA TAG CAC TCT CTC CTA TTA AAG TAG G (SEQ ID NO: 208) into the 5' NdeI/3' KPN cloning site of the vector. The carboxy terminus portion of the gene, pmpCcarboxy terminal fragment (SEQ ID NO: 186, with the corresponding amino acid sequence provided in SEQ ID NO: 194), was subcloned into the 5' NheI/3' KPN cloning site of the expression vector using the following primers: 5' oligo- CAG AGC TAG CAT GCA TCA CCA TCA CCA TCA CGT TAA GAT TGA GAA CTT CTC TGG C (SEQ ID NO: 209), and 3' oligo- CAG AGG TAC CTT AGA ATG TCA TAC GAG CAC CGC AG (SEQ ID PmpD was also expressed as two overlapping proteins. The pmpD-amino NO: 210). terminal portion, which lacks the signal sequence, (SEQ ID NO: 185, with the corresponding amino acid sequence provided in SEQ ID NO: 193) contains the initiating codon of the pET17b and is expressed as a 80 kD protein. For protein expression and purification purposes, a six-histidine tag follows the initiation codon and is fused at the 28th amino acid (nucleotide 84) of the gene. The following primers were used, 5' oligo, CAG ACA TAT GCA TCA CCA TCA CGG GTT AGC (SEQ ID NO: 211), and the 3' oligo-CAG AGG TAC CTC AGC TCC AGC ACA CTC TCT TC (SEQ ID NO: 212), to splice into the 5' NdeI/3' KPN cloning site of the vector. The pmpD-carboxy terminus portion (SEQ ID NO: 184) was expressed as a 92 kD protein (SEQ ID NO: 192). For expression and subsequent purification, an additional methionine, alanine and serine was included, which represent the initiation codon and the first two amino acids from the pET17b vector. A six-histidine tag downstream of the methionine, alanine and serine is fused at the 691st amino acid (nucleotide 2073) of the gene. The 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA CGG TGC TAT TTC TTG CTT ACG TGG (SEQ ID NO: 213) and the 3' oligo- CAG AGG TAC TTn AAA AGA TCA ATC GCA ATC CAG TAT TCG (SEQ ID NO: 214) were used to subclone the insert into the 5' NheI/3' KPN cloning site of the expression vector. PmpE was expressed as a 106kD protein (SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191). The pmpE insert also lacks the native signal sequence. PCR amplification of the gene under conditions well known in the art was performed using the following oligo primers: 5' oligo- CAG AGG ATC CAC ATC ACC ATC ACC ATC ACG GAC TAG CTA GAG AGG TTC (SEQ ID NO: 215), and

the 3' oligo- CAG AGA ATT CCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 216), and the amplified insert was ligated into a 5' BamHI/3' EcoRI site of JA4304. The short nucleotide sequence, as provided in SEQ ID NO: 217, was inserted upstream of the initiation codon for creating the Kozak-like sequence and reconstituting the HindIII site. expressed protein contains the initiation codon and the downstream 21 amino acids from the pET17b expression vector, i.e., MASMTGGQQMGRDSSLVPSSDP (SEQ ID NO: 218). In addition, a six-histidine tag is included upstream of the sequence described above and is fused at the 28th amino acid (nucleotide 84) of the gene, which eliminates the hypothetical signal peptide. The sequences provided in SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191 do not include these additional sequences. The pmpG gene (SEQ ID NO: 182, with the corresponding amino acid sequence provided in SEQ ID No; 190) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo- CAG AGG TAC CGC ATC ACC ATC ACC ATC ACA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 219), and the 3' oligo- CAG AGC GGC CGC TTA GAA CCG GAC TTT ACT TCC (SEQ ID NO: 220), and ligated into the 5' KPN/3' NotI cloning site of the expression vector. The expressed protein contains an additional amino acid sequence at the amino end, namely, MASMTGGQQNGRDSSLVPHHHHHHH (SEQ ID NO: 221), which comprises the initiation codon and additional sequence from the pET17b expression vector. The pmpI gene (SEQ ID NO: 181, with the corresponding amino acid sequence provided in SEQ ID No; 189) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA CCT CTT TGG CCA GGA TCC C (SEQ ID NO: 222), and the 3' oligo- CAG AAC TAG TCT AGA ACC TGT AAG TGG TCC (SEQ ID NO: 223), and ligted into the expression vector at the 5' NheI/3' SpeI cloning site. The 95 kD expressed protein contains the initiation codon plus an additional alanine and serine from the pET17b vector at the amino end of the protein. In addition, a six-histidine tag is fused at the 21st amino acid of the gene, which eliminates the hypothetical signal peptide.

Clone 14H1-4, (SEQ ID NO: 56), identified using the TCT-3 cell line, contains a complete ORF for the TSA gene, thiol specific antioxidant – CT603 (the CT603 ORF is a homolog of CPn0778 from *C. pnuemoniae*). The TSA open reading frame in clone

14-H1-4 was amplified such that the expressed protein possess an additional methionine and a 6x histidine tag (amino terminal end). This amplified insert was sub-cloned into the Nde/EcoRI sites of the pET17b vector. Upon induction of this clone with IPTG, a 22.6 kDa protein was purified by Ni-NTA agarose affinity chromatography. The determined amino acid sequence for the 195 amino acid ORF of clone 14-H1-4 encoding the TSA gene is provided in SEQ ID NO: 65. Further analysis yielded a full-length clone for the TSA gene, referred to as CTL2-TSA-FL, with the full-length amino acid sequence provided in SEQ ID NO: 92.

Further studies yielded 10 additional clones identified by the TCT-1 and TCT-3 T-cell lines, as described above. The clones identified by the TCT-1 line are: 16-D4-22, 17-C5-19, 18-C5-2, 20-G3-45 and 21-C7-66; clones identified by the TCT-3 cell line are: 17-C10-31, 17-E2-9, 22-A1-49 and 22-B3-53. Clone 21-G12-60 was recognized by both the TCT-1 and TCT-3 T cell lines. Clone 16-D4-22 (SEQ ID NO: 119), identified using the TCT-1 cell line contains a 953 bp insert that contains two genes, parts of open reading frame 3 (ORF3) and ORF4 of the C. trachomatis plasmid for growth within mammalian cells. Clone 17-C5-19 (SEQ ID NO: 118), contains a 951 bp insert that contains part of the ORF for DT431, encoding for clpP_1 protease and part of the ORF for CT430 (diaminopimelate epimerase). Clone 18-C5-2 (SEQ ID NO: 117) is part of the ORF for S1 ribosomal protein with a 446 bp insert that was identified using the TCT-1 cell line. Clone 20-G3-45 (SEQ ID NO: 116), identified by the TCT-1 cell line, contains a 437 bp insert that is part of the pmpB gene (CT413). Clone 21-C7-66 (SEQ ID NO: 115), identified by the TCT-1 line, contains a 995bp insert that encodes part of the dnaK like protein. The insert of this clone does not overlap with the insert of the TCT-3 clone 11-H4-28 (SEQ ID NO: 59), which was shown to be part of the dnaK gene CT396 Clone 17-C10-31 (SEQ ID NO: 114), identified by the TCT-3 cell line, contains a 976 bp insert. This clone contains part of the ORF for CT858, a protease containing IRBP and DHR domains. Clone 17-E2-9 (SEQ ID NO: 113) contains part of ORFs for two genes, CT611 and CT610, that span a 1142 bp insert. Clone 22-A1-49 (SEQ ID NO: 112), identified using the TCT-3 line, also contains two genes in a 698 bp insert. Part of the ORF for CT660 (DNA gyrase{gyrA_2}) is present on the top strand where as the complete ORF for a hypothetical protein CT659 is present on the complementary

strand. Clone 22-B3-53 (SEQ ID NO: 111), identified by the TCT-1 line, has a 267 bp insert that encodes part of the ORF for GroEL (CT110). Clone 21-G12-60 (SEQ ID NO: 110), identified by both the TCT-1 and TCT-3 cell lines contains a 1461 bp insert that contains partial ORFs for hypothetical proteins CT875, CT229 and CT228.

Additional Chlamydia antigens were obtained by screening a genomic expression library of Chlamydia trachomatis (LGV II serovar) in Lambda Screen-1 vector (Novagen, Madison, WI) with sera pooled from several Chlamydia-infected individuals using techniques well known in the art. The following immuno-reactive clones were identified and the inserts containing Chlamydia genes sequenced: CTL2#1 (SEQ ID NO: 71); CTL2#2 (SEQ ID NO: 70); CTL2#3-5' (SEQ ID NO: 72, a first determined genomic sequence representing the 5' end); CTL2#3-3' (SEQ ID NO: 73, a second determined genomic sequence representing the 3' end); CTL2#4 (SEQ ID NO: 53); CTL2#5 (SEQ ID NO: 69); CTL2#6 (SEQ ID NO: 68); CTL2#7 (SEQ ID NO: 67); CTL2#8b (SEQ ID NO: 54); CTL2#9 (SEQ ID NO: 66); CTL2#10-5' (SEQ ID NO: 74, a first determined genomic sequence representing the 5' end); CTL2#10-3' (SEQ ID NO: 75, a second determined genomic sequence representing the 3' end); CTL2#11-5' (SEQ ID NO: 45, a first determined genomic sequence representing the 5' end); CTL2#11-3' (SEQ ID NO: 44, a second determined genomic sequence representing the 3' end); CTL2#12 (SEQ ID NO: 46); CTL2#16-5' (SEQ ID NO: 47); CTL2#18-5' (SEQ ID NO: 49, a first determined genomic sequence representing the 5' end); CTL2#18-3' (SEQ ID NO: 48, a second determined genomic sequence representing the 3' end); CTL2#19-5' (SEQ ID NO: 76, the determined genomic sequence representing the 5' end); CTL2#21 (SEQ ID NO: 50); CTL2#23 (SEQ ID NO: 51; and CTL2#24 (SEQ ID NO: 52).

Additional *Chlamydia trachomatis* antigens were identified by serological expression cloning. These studies used sera pooled from several *Chlamydia*-infected individuals, as described above, but, IgA, and IgM antibodies were used in addition to IgG as a secondary antibody. Clones screened by this method enhance detection of antigens recognized by an early immune response to a *Chlamydial* infection, that is a mucosal humoral immune response. The following immunoreactive clones were characterized and the inserts containing *Chlamydia* genes sequenced: CTL2gam-1 (SEQ ID NO: 290), CTL2gam-2 (SEQ

ID NO: 289), CTL2gam-5 (SEQ ID NO: 288), CTL2gam-6-3' (SEQ ID NO: 287, a second determined genomic sequence representing the 3' end), CTL2gam-6-5' (SEQ ID NO: 286, a first determined genomic sequence representing the 5' end), CTL2gam-8 (SEQ ID NO: 285), CTL2gam-10 (SEQ ID NO: 284), CTL2gam-13 (SEQ ID NO: 283), CTL2gam-15-3' (SEQ ID NO: 282, a second determined genomic sequence representing the 3' end), CTL2gam-15-5' (SEQ ID NO: 281, a first determined genomic sequence representing the 5' end), CTL2gam-17 (SEQ ID NO: 280), CTL2gam-18 (SEQ ID NO: 279), CTL2gam-21 (SEQ ID NO: 278), CTL2gam-23 (SEQ ID NO: 277), CTL2gam-24 (SEQ ID NO: 276), CTL2gam-26 (SEQ ID NO: 275), CTL2gam-27 (SEQ ID NO: 274), CTL2gam-28 (SEQ ID NO: 273), CTL2gam-30-3' (SEQ ID NO: 272, a second determined genomic sequence representing the 3' end) and CTL2gam-30-5' (SEQ ID NO: 271, a first determined genomic sequence representing the 5' end).

EXAMPLE 2

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y PRODUCTION BY CHLAMYDIA TRACHOMATIS ANTIGENS

The ability of recombinant *Chlamydia trachomatis* antigens to induce T cell proliferation and interferon-γ production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatograph (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. trachomatis* patients as well as from normal donors whose T-cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μ g/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 μ g/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μ l, 50 μ l of medium is removed from each well for determination of IFN- γ levels, as described below. The plates are then pulsed with 1 μ Ci/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that

result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ is measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

Using the above methodology, recombinant 1B1-66 protein (SEQ ID NO: 5) as well as two synthetic peptides corresponding to amino acid residues 48-67 (SEQ ID NO: 13; referred to as 1-B1-66/48-67) and 58-77 (SEQ ID NO: 14, referred to as 1B1-66/58-77), respectively, of SEQ ID NO: 5, were found to induce a proliferative response and IFN-γ production in a Chlamydia-specific T cell line used to screen a genomic library of C. trachomatis LGV II.

Further studies have identified a *C. trachomatis*-specific T-cell epitope in the ribosomal S13 protein. Employing standard epitope mapping techniques well known in the art, two T-cell epitopes in the ribosomal S13 protein (rS13) were identified with a *Chlamydia*-specific T-cell line from donor CL-8 (T-cell line TCL-8 EB/DC). Fig. 8 illustrates that the first peptide, rS13 1-20 (SEQ ID NO: 106), is 100% identical with the corresponding *C. pneumoniae* sequence, explaining the cross-reactivity of the T-cell line to recombinant *C. trachomatis*- and *C. pneumoniae*-rS13. The response to the second peptide

rS13 56-75 (SEQ ID NO: 108) is *C. trachomatis*-specific, indicating that the rS13 response in this healthy asymptomatic donor was elicited by exposure to *C. trachomatis* and not to *C. pneumoniae*, or any other microbial infection.

As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10⁴ TCP-21 T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-21 T-cell line also gave a proliferative response to the homologous C. pneumoniae peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the C. trachomatis peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between C. trachomatis and C. pneumoniae.

To further define the epitope described above, an additional T-cell line, TCT-3, was used in epitope mapping experiments. The immunoassays were performed as described above, except that only peptides from *C. trachomatis* were tested. The T-cells gave a proliferative response to two peptides, CT-OMCB #152-171 and CT-OMCB #157-176 (SEQ ID NO: 246 and 247, respectively), thereby defining an additional immunogenic epitope in the cysteine rich outer membrane protein of *C. trachomatis*.

Clone 14H1-4, (SEQ ID NO: 56, with the corresponding full-length amino acid sequence provided in SEQ ID NO: 92), was identified using the TCT-3 cell line in the CD4 T-cell expression cloning system previously described, and was shown to contain a complete ORF for the, thiol specific antioxidant gene (CT603), referred to as TSA. Epitope

mapping immunoassays were performed, as described above, to further define the epitope. The TCT-3 T-cells line exhibited a strong proliferative response to the overlapping peptides CT-TSA #96-115, CT-TSA #101-120 and CT-TSA #106-125 (SEQ ID NO: 254-256, respectively) demonstrating an immunoreactive epitope in the thiol specific antioxidant gene of *C. trachomatis* serovar LGVII.

EXAMPLE 3 PREPARATION OF SYNTHETIC POLYPEPTIDES

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugating or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray mass spectrometry and by amino acid analysis.

EXAMPLE 4

ISOLATION AND CHARACTERIZATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS USING RETROVIRAL EXPRESSION VECTOR SYSTEMS AND SUBSEQUENT IMMUNOLOGICAL ANALYSIS

A genomic library of *Chlamydia trachomatis* LGV II was constructed by limited digests using BamHI, BglII, BstYi and MboI restriction enzymes. The restriction digest fragments were subsequently ligated into the BamHI site of the retroviral vectors pBIB-KS1,2,3. This vector set was modified to contain a Kosak translation initiation site and stop codons in order to allow expression of proteins from short DNA genomic fragments, as shown in Fig. 2. DNA pools of 80 clones were prepared and transfected into the retroviral packaging line Phoenix-Ampho, as described in Pear, W.S., Scott, M.L. and Nolan, G.P., Generation of High Titre, Helper-free Retroviruses by Transient Transfection. Methods in Molecular Medicine: Gene Therapy Protocols, Humana Press, Totowa, NJ, pp. 41-57. The *Chlamydia* library in retroviral form was then transduced into H2-Ld expressing P815 cells, which were then used as target cells to stimulate an antigen specific T-cell line.

A Chlamydia-specific, murine H2^d restricted CD8+ T-cell line was expanded in culture by repeated rounds of stimulation with irradiated C. trachomatis-infected J774 cells and irradiated syngeneic spleen cells, as described by Starnbach, M., in J. Immunol., 153:5183, 1994. This Chlamydia-specific T-cell line was used to screen the above Chlamydia genomic library expressed by the retrovirally-transduced P815 cells. Positive DNA pools were identified by detection of IFN-γ production using Elispot analysis (SEE Lalvani et al., J. Experimental Medicine 186:859-865, 1997).

Two positive pools, referred to as 2C7 and 2E10, were identified by IFN-γ Elispot assays. Stable transductants of P815 cells from pool 2C7 were cloned by limiting dilution and individual clones were selected based upon their capacity to elicit IFN-γ production from the *Chlamydia*-specific CTL line. From this screening process, four positive clones were selected, referred to as 2C7-8, 2C7-9, 2C7-19 and 2C7-21. Similarly, the positive pool 2E10 was further screened, resulting in a an additional positive clone, which

contains three inserts. The three inserts are fragments of the CT016, tRNA syntase and clpX genes (SEQ ID NO: 268-270, respectively).

Transgenic DNA from these four positive 2C7.8 clones were PCR amplified using pBIB-KS specific primers to selectively amplify the *Chlamydia* DNA insert. Amplified inserts were gel purified and sequenced. One immunoreactive clone, 2C7-8 (SEQ ID NO: 15, with the predicted amino acid sequence provided in SEQ ID NO: 32), is a 160 bp fragment with homology to nucleotides 597304-597145 of *Chlamydia trachomatis*, serovar D (NCBI, BLASTN search; SEQ ID NO: 33, with the predicted amino acid sequence provided in SEQ ID NO: 34). The sequence of clone 2C7-8 maps within two putative open reading frames from the region of high homology described immediately above, and in particular, one of these putative open reading frames, consisting of a 298 amino acid fragment (SEQ ID NO: 16, with the predicted amino acid sequence provided in SEQ ID NO: 17), was demonstrated to exhibit immunological activity.

Full-length cloning of the 298 amino acid fragment (referred to as CT529 and/or the Cap1 gene) from serovar L2 was obtained by PCR amplification using 5'-ttttgaagcaggtaggtgaatatg (forward) (SEQ ID NO: 159) and 5'-ttaagaaatttaaaaaatccctta (reverse) (SEQ ID NO: 160) primers, using purified *C. trachomatis* L2 genomic DNA as template. This PCR product was gel-purified, cloned into pCRBlunt (Invitrogen, Carlsbad, CA) for sequencing, and then subcloned into the *Eco*RI site of pBIB-KMS, a derivative of pBIB-KS for expression. The *Chlamydia pnuemoniae* homlogue of CT529 is provided in SEQ ID NO: 291, with the corresponding amino acid sequence provided in SEQ ID NO: 292.

Full-length DNA encoding various CT529 serovars were amplified by PCR from bacterial lysates containing 10⁵ IFU, essentially as described (Denamur, E., C. Sayada, A. Souriau, J. Orfila, A. Rodolakis and J. Elion. 1991. J. Gen. Microbiol. 137: 2525). The following serovars were amplified as described: Ba (SEQ ID NO: 134, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 135); E (BOUR) and E (MTW447) (SEQ ID NO: 122, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 123); F (NI1) (SEQ ID NO: 128, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 126, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 127); Ia (SEQ ID

NO: 124, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 125); L1 (SEQ ID NO: 130, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 131); L3 (SEQ ID NO: 132, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 133); I (SEQ ID NO: 263, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 264); K (SEQ ID NO: 265, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 266); and MoPn (SEQ ID NO: 136, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 137). PCR reactions were performed with Advantage Genomic PCR Kit (Clontech, Palo Alto, CA) using primers specific for serovar L2 DNA (external to the ORF). Primers sequences were 5'-ggtataatatctctaaattttg (forward-SEQ ID NO: 161) and 5'-agataaaaaaaggctgtttc' (reverse-SEQ ID NO: 162) except for MoPn which required 5'-ttttgaagcaggtaggtgaatatg (forward-SEQ ID NO: 163) and 5'-tttacaataagaaaagctaagcactttgt (reverse-SEQ ID NO: 164). PCR amplified DNA was purified with QIAquick PCR purification kit (Qiagen, Valencia, CA) and cloned in pCR2.1 (Invitrogen, Carlsbad, CA) for sequencing.

Sequencing of DNA derived from PCR amplified inserts of immunoreactive clones was done on an automated sequencer (ABI 377) using both a pBIB-KS specific forward primer 5'-cettacacagtcctgctgac (SEQ ID NO: 165) and a reverse primer 3'-gtttccgggccctcacattg (SEQ ID NO: 166). PCRBlunt cloned DNA coding for CT529 serovar L2 and pCR2.1 cloned DNA coding for CT529 serovar Ba, E (BOUR), E (MTW447), F (NI1), G, Ia, K, L1, L3 and MoPn were sequenced using T7 promoter primer and universal M13 forward and M13 reverse primers.

To determine if these two putative open reading frames (SEQ ID NO: 16 and 20) encoded a protein with an associated immunological function, overlapping peptides (17-20 amino acid lengths) spanning the lengths of the two open reading frames were synthesized, as described in Example 3. A standard chromium release assay was utilized to determine the per cent specific lysis of peptide-pulsed H2^d restricted target cells. In this assay, aliquots of P815 cells (H2^d) were labeled at 37° C for one hour with 100 μ Ci of ⁵¹Cr in the presence or absence of 1 μ g/ml of the indicated peptides. Following this incubation, labeled P815 cells were washed to remove excess ⁵¹Cr and peptide, and subsequently plated

in duplicate in microculture plates at a concentration of 1,000 cells/well. Effector CTL (Chlamydia-specific CD8 T cells) were added at the indicated effector:target ratios. Following a 4 hour incubation, supernatants were harvested and measured by gamma-counter for release of ⁵¹Cr into the supernatant. Two overlapping peptides from the 298 amino acid open reading frame did specifically stimulate the CTL line. The peptides represented in SEQ ID NO: 138-156 were synthsized, representing the translation of the L2 homologue of the serovar D open reading frame for CT529 (Cap1 gene) and 216 amino acid open reading frame. As shown in Fig. 3, peptides CtC7.8-12 (SEQ ID NO: 18, also referred to as Cap1#132-147, SEQ ID NO: 139) and CtC7.8-13 (SEQ ID NO: 19, also referred to as Cap1#138-155, SEQ ID NO: 140) were able to elicit 38 to 52% specific lysis, respectively, at an effector to target ratio of 10:1. Notably, the overlap between these two peptides contained a predicted H2^d (K^d and L^d) binding peptide. A 10 amino acid peptide was synthesized to correspond to this overlapping sequence (SEQ ID NO: 31) and was found to generate a strong immune response from the anti-Chlamydia CTL line by elispot assay. Significantly, a search of the most recent Genbank database revealed no proteins have previously been described for this gene. Therefore, the putative open reading frame encoding clone 2C7-8 (SEQ ID NO: 15) defines a gene which encompasses an antigen from Chlamydia capable of stimulating antigen-specific CD8+ T-cells in a MHC-I restricted manner, demonstrating this antigen could be used to develop a vaccine against Chlamydia.

To confirm these results and to further map the epitope, truncated peptides (SEQ ID NO: 138-156) were made and tested for recognition by the T-cells in an IFN-g ELISPOT assay. Truncations of either Ser139 (Cap1#140-147, SEQ ID NO: 146) or Leu147 (Cap1#138-146, SEQ ID NO: 147) abrogate T-cell recognition. These results indicate that the 9-mer peptide Cap1#139-147 (SFIGGITYL, SEQ ID NO: 145) is the minimal epitope recognized by the *Chlamydia*-specific T-cells.

Sequence alignments of Cap1 (CT529) from selected serovars of *C. trachomatis* (SEQ ID NO: 121, 123, 125, 127, 129, 131, 133, 135, 137 and 139) shows one of the amino acid differences is found in position 2 of the proposed epitope. The homologous serovar D peptide is SIIGGITYL (SEQ ID NO: 168). The ability of SFIGGITYL and SIIGGITYL to target cells for recognition by the *Chlamydia* specific T-cells was compared.

Serial dilutions of each peptide were incubated with P815 cells and tested for recognition by the T-cells in a ⁵¹Cr release assay, as described above. The *Chlamydia*-specific T-cells recognize the serovar L2 peptide at a minimum concentration of 1 nM and the serovar D peptide at a minimum concentration of 10 nM.

Further studies have shown that a Cap1#139-147-specific T-cell clone recognizes *C. trachomatis* infected cells. To confirm that Cap1₁₃₉₋₁₄₇ is presented on the surface of *Chlamydia* infected cells, Balb-3T3 (H-2^d) cells were infected with *C. trachomatis* scrovar L2 and tested to determine whether these cells are recognized by a CD8+ T-cell clone specific for Cap1#139-147 epitope (SEQ ID NO: 145). The T-cell clone specific for Cap1#139-147 epitope was obtained by limiting dilution of the line 69 T-cells. The T-cell clone specifically recognized the *Chlamydia* infected cells. In these experiments, target cells were *C. trachomatis* infected (positive control) or uninfected Balb/3T3 cells, showing 45%, 36% and 30% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively; or Cap1#139-147 epitope (SEQ ID NO: 145) coated, or untreated P815 cells, showing 83%, 75% and 58% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively (negative controls having less than 5% lysis in all cases). This data suggests that the epitope is presented during infection.

In vivo studies show Cap1#139-147 epitope-specific T-cells are primed during murine infection with *C. trachomatis*. To determine if infection with *C. trachomatis* primes a Cap1#139-147 epitope-specific T-cell response, mice were infected i.p. with 10⁸ IFU of *C. trachomatis* serovar L2. Two weeks after infection, the mice were sacrificed and spleen cells were stimulated on irradiated syngeneic spleen cells pulsed with Cap1#139-147 epitope peptide. After 5 days of stimulation, the cultures were used in a standard ⁵¹Cr release assay to determine if there were Cap1#139-147 epitope-specific T-cells present in the culture. Specifically, spleen cells from a *C. trachomatis* serovar L2 immunized mouse or a control mouse injected with PBS after a 5 days culture with Cap1#139-147 peptide-coated syngeneic spleen cells and CD8+ T-cells able to specifically recognize Cap1#139-147 epitope gave 73%, 60% and 32% specific lysis at a30:1, 10:1 and 3:1 effector to target ratios, respectively. The control mice had a percent lysis of approximately 10% at a 30:1 effector to target ratio, and steadily declining with lowering E:T ratios. Target cells were Cap1#139-147 peptide-

coated, or untreated P815 cells. These data suggest that Cap1#139-147 peptide-specific T-cells are primed during murine infection with *C. trachomatis*.

EXAMPLE 5

GENERATION OF ANTIBODY AND T-CELL RESPONSES IN MICE IMMUNIZED WITH CHLAMYDIA ANTIGENS

Immunogenicity studies were conducted to determine the antibody and CD4+ T cell responses in mice immunized with either purified SWIB or S13 proteins formulated with Montanide adjuvant, or DNA-based immunizations with pcDNA-3 expression vectors containing the DNA sequences for SWIB or S13. SWIB is also referred to as clone 1-B1-66 (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5), and S13 ribosomal protein is also referred to as clone 10-C10-31 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12). In the first experiment, groups of three C57BL/6 mice were immunized twice and monitored for antibody and CD4+ T-cell responses. DNA immunizations were intradermal at the base of the tail and polypeptide immunizations were administered by subcutaneous route. Results from standard ³H-incorporation assays of spleen cells from immunized mice shows a strong proliferative response from the group immunized with purified recombinant SWIB polypeptide (SEQ ID NO: 5). Further analysis by cytokine induction assays, as previously described, demonstrated that the group immunized with SWIB polypeptide produced a measurable IFN-y and IL-4 response. Subsequent ELISA-based assays to determine the predominant antibody isotype response in the experimental group immunized with the SWIB polypeptide were performed. Fig. 4 illustrates the SWIB-immunized group gave a humoral response that was predominantly IgG1.

In a second experiment, C3H mice were immunized three times with 10 μ g purified SWIB protein (also referred to as clone 1-B1-66, SEQ ID NO: 5) formulated in either PBS or Montanide at three week intervals and harvested two weeks after the third immunization. Antibody titers directed against the SWIB protein were determined by

standard ELISA-based techniques well known in the art, demonstrating the SWIB protein formulated with Montanide adjuvant induced a strong humoral immune response. T-cell proliferative responses were determined by a XTT-based assay (Scudiero, et al, *Cancer Research*, 1988, 48:4827). As shown in Fig. 5, splenocytes from mice immunized with the SWIB polypeptide plus Montanide elicited an antigen specific proliferative response. In addition, the capacity of splenocytes from immunized animals to secrete IFN-γ in response to soluble recombinant SWIB polypeptide was determined using the cytokine induction assay previously described. The splenocytes from all animals in the group immunized with SWIB polypeptide formulated with montanide adjuvant secreted IFN-γ in response to exposure to the SWIB Chlamydia antigen, demonstrating an *Chlamydia*-specific immune response.

In a further experiment, C3H mice were immunized at three separate time points at the base of the tail with 10 μg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) formulated with the SBAS2 adjuvant (SmithKline Beecham, London, England). Antigen-specific antibody titers were measured by ELISA, showing both polypeptides induced a strong IgG response, ranging in titers from 1 x10⁻⁴ to 1 x10⁻⁵. The IgG1 and IgG2a components of this response were present in fairly equal amounts. Antigen-specific T-cell proliferative responses, determined by standard ³H-incorporation assays on spleen cells isolated from immunized mice, were quite strong for SWIB (50,000 cpm above the negative control) and even stronger for s13 (100,000 cpm above the negative control). The IFNγ production was assayed by standard ELISA techniques from supernatant from the proliferating culture. *In vitro* restimulation of the culture with S13 protein induced high levels of IFNγ production, approximately 25 ng/ml versus 2 ng/ml for the negative control. Restimulation with the SWIB protein also induced IFNγ, although to a lesser extent.

In a related experiment, C3H mice were immunized at three separate time points with 10 μg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) mixed with 10 μg of Cholera Toxin. Mucosal immunization was through intranasal inoculation. Antigenspecific antibody responses were determined by standard ELISA techniques. Antigenspecific IgG antibodies were present in the blood of SWIB-immunized mice, with titers

ranging from 1 x10⁻³ to 1 x10⁻⁴, but non-detectable in the S13-immunized animals. Antigen-specific T-cell responses from isolated splenocytes, as measured by IFNy production, gave similar results to those described immediately above for systemic immunization.

An animal study was conducted to determine the immunogenicity of the CT529 serovar LGVII CTL epitope, defined by the CT529 10mer consensus peptide (CSFIGGITYL - SEQ ID NO: 31), which was identified as an H2-Kd restricted CTL epitope. BALB/c mice (3 mice per group) were immunized three times with 25 μg of peptide combined with various adjuvants. The peptide was administered systemically at the base of the tail in either SKB Adjuvant System SBAS-2", SBAS-7 (SmithKline Beecham, London, England) or Montanide. The peptide was also administered intranasally mixed with 10ug of Cholera Toxin (CT). Naive mice were used as a control. Four weeks after the 3rd immunization, spleen cells were restimulated with LPS-blasts pulsed with 10ug/ml CT529 10mer consensus peptide at three different effector to LPS-blasts ratios: 6, 1.5 and 0.4 at 1x106 cell/ml. After 2 restimulations, effector cells were tested for their ability to lyse peptide pulsed P815 cells using a standard chromium release assay. A non-relevant peptide from chicken egg ovalbumin was used as a negative control. The results demonstrate that a significant immune response was elicited towards the CT529 10mer consensus peptide and that antigen-specific T-cells capable of lysing peptide-pulsed targets were elicited in response to immunization with the peptide. Specifically, antigen-specific lytic activities were found in the SBAS-7 and CT adjuvanted group while Montanide and SBAS-2" failed to adjuvant the CTL epitope immunization.

EXAMPLE 6

EXPRESSION AND CHARACTERIZATION OF CHLAMYDIA PNEUMONIAE GENES

The human T-cell line, TCL-8, described in Example 1, recognizes Chlamydia trachomatis as well as Chlamydia pneumonia infected monocyte-derived dendritic cells, suggesting Chlamydia trachomatis and pneumonia may encode cross-reactive T-cell epitopes. To isolate the Chlamydia pneumonia genes homologous to Chlamydia trachomatis LGV II

clones 1B1-66, also referred to as SWIB (SEQ ID NO: 1) and clone 10C10-31, also referred to as S13 ribosomal protein (SEQ ID NO: 4), HeLa 229 cells were infected with *C. pneumonia* strain TWAR (CDC/CWL-029). After three days incubation, the *C. pneumonia*-infected HeLa cells were harvested, washed and resuspended in 200 µl water and heated in a boiling water bath for 20 minutes. Ten microliters of the disrupted cell suspension was used as the PCR template.

C. pneumonia specific primers were designed for clones 1B1-66 and 10C10-31 such that the 5' end had a 6X-Histidine tag and a Nde I site inserted, and the 3' end had a stop codon and a BamHI site included (Fig. 6). The PCR products were amplified and sequenced by standard techniques well known in the art. The C. pneumonia-specific PCR products were cloned into expression vector pET17B (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Two proteins from C. pneumonia were thus generated, a 10-11 kDa protein referred to as CpSWIB (SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 30 and 91, respectively).

EXAMPLE 7

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y PRODUCTION BY CHLAMYDIA PNEUMONIAE ANTIGENS

The ability of recombinant *Chlamydia pneumoniae* antigens to induce T cell proliferation and interferon-y production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatography (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. pneumoniae* patients as well as from normal donors whose T-

cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μ g/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 μ g/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μ l, 50 μ l of medium is removed from each well for determination of IFN- γ levels, as described below. The plates are then pulsed with 1 μ Ci/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

A human anti-Chlamydia T-cell line (TCL-8) capable of cross-reacting to C. trachomatis and C. pneumonia was used to determine whether the expressed proteins described in the example above, (i.e., CpSWIB, SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 28, respectively, and the 15 kDa protein referred to as CpS13 SEQ ID NO: 29, and SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID

NO: 30 and 91, respectively), possessed T-cell epitopes common to both C. trachomatis and C. pneumonia. Briefly, E. coli expressing Chlamydial proteins were titered on 1 x 104 monocyte-derived dendritic cells. After two hours, the dendritic_cells cultures were washed and 2.5 x 10⁴ T cells (TCL-8) added and allowed to incubate for an additional 72 hours. The amount of INF-γ in the culture supernatant was then determined by ELISA. As shown in Figs. 7A and 7B, the TCL-8 T-cell line specifically recognized the S13 ribosomal protein from both C. trachomatis and C. pneumonia as demonstrated by the antigen-specific induction of IFN-γ, whereas only the SWIB protein from C. trachomatis was recognized by the T-cell line. To validate these results, the T cell epitope of C. trachomatis SWIB was identified by epitope mapping using target cells pulsed with a series of overlapping peptides and the T-cell line TCL-8. 3H-thymidine incorporation assays demonstrated that the peptide, referred to as C.t.SWIB 52-67, of SEQ ID NO: 39 gave the strongest proliferation of the TCL-8 line. The homologous peptides corresponding to the SWIB of C. pneumoniae sequence (SEQ ID NO: 40), the topoisomerase-SWIB fusion of C. pneumoniae (SEQ ID NO: 43) and C. trachomatis (SEQ ID NO: 42) as well as the human SWI domain (SEQ ID NO: 41) were synthesized and tested in the above assay. The T-cell line TCL-8 only recognized the C. trachomatis peptide of SEQ ID NO: 39 and not the corresponding C. pneumoniae peptide (SEQ ID NO: 40), or the other corresponding peptides described above (SEQ ID NO; 41-43).

Chlamydia-specific T cell lines were generated from donor CP-21 with a positive serum titer against *C. pneumoniae* by stimulating donor PBMC with either *C. trachomatis* or *C. pneumoniae*-infected monocyte-derived dendritic cells, respectively. T-cells generated against *C. pneumoniae* responded to recombinant *C. pneumoniae*-SWIB but not *C. trachomatis*-SWIB, whereas the T-cell line generated against *C. trachomatis* did not respond to either *C. trachomatis*- or *C. pneumoniae*-SWIB (see Fig. 9). The *C. pneumoniae*-SWIB specific immune response of donor CP-21 confirms the *C. pneumoniae* infection and indicates the elicitation of *C. pneumoniae*-SWIB specific T-cells during *in vivo C. pneumoniae* infection.

Epitope mapping of the T-cell response to *C. pneumoniae*-SWIB has shown that Cp-SWIB-specific T-cells responded to the overlapping peptides Cp-SWIB 32-51 (SEQ

ID NO: 101) and Cp-SWIB 37-56 (SEQ ID NO: 102), indicating a *C. pneumoniae*-SWIB-specific T-cell epitope Cp-SWIB 37-51 (SEQ ID NO: 100).

In additional experiments, T-cell lines were generated from donor CP1, also a C. pneumoniae seropositive donor, by stimulating PBMC with non-infectious elementary bodies from C. trachomatis and C. pneumoniae, respectively. In particular, proliferative responses were determined by stimulating 2.5 x 10⁴ T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells and non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or either recombinant C. trachomatis or C. pneumoniae SWIB protein. The T-cell response against SWIB resembled the data obtained with T-cell lines from CP-21 in that C. pneumoniae-SWIB, but not C. trachomatis-SWIB elicited a response by the C. pneumoniae T-cell line. In addition, the C. trachomatis T-cell line did not proliferate in response to either C. trachomatis or C. pneumoniae SWIB, though it did proliferate in response to both CT and CP elementary bodies. As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10⁴ TCP-21 T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-21 T-cell line also gave a proliferative response to the homologous C. pneumoniae peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the to the C. trachomatis peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between C. trachomatis and C. pneumoniae.

EXAMPLE 8

IMMUNE RESPONSES OF HUMAN PBMC AND T-CELL LINES AGAINST CHLAMYDIA ANTIGENS

The examples provided herein suggest that there is a population of healthy donors among the general population that have been infected with C. trachomatis and generated a protective immune response controlling the C. trachomatis infection. These donors remained clinically asymptomatic and seronegative for C. trachomatis. To characterize the immune responses of normal donors against chlamydial antigens which had been identified by CD4 expression cloning, PBMC obtained from 12 healthy donors were tested against a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and C. trachomatis-, C. pneumoniae-S13. The data are summarized in Table I below. All donors were seronegative for C. trachomatis, whereas 6/12 had a positive C. pneumoniae titer. Using a stimulation index of >4 as a positive response, 11/12 of the subjects responded to C. trachomatis elementary bodies and 12/12 responded to C. pneumoniae elementary bodies. One donor, AD104, responded to recombinant C. pneumoniae-S13 protein, but not to recombinant C. trachomatis-S13 protein, indicating a C. pneumoniae-specific response. Three out of 12 donors had a C. trachomatis-SWIB, but not a C. pneumoniae-SWIB specific response, confirming a C. trachomatis infection. C. trachomatis and C. pneumoniae- S13 elicited a response in 8/12 donors suggesting a chlamydial infection. These data demonstrate the ability of SWIB and S13 to elicit a T-cell response in PBMC of normal study subjects.

Table I.

Immune response of normal study subjects against Changdia

onor	Sex	<i>Chlamydia</i> IgGtiter	CT EB	CP EB	CT Swib	CP Swib	CI SI3	CP SI3	Cl lpdA	CT TSA
D100	male	negative	++	+++	+	-	++	++	-	nt.
D104	female	negative	+++	++	-	-	-	++	-	nt
D108	male	CP 1:256	++	++	+	+/-	+	+	+	nt.
D112	female	negative	++	++	+	-	+	-	+/-	nt
D120	male	negative	-	+	-	-	-	-	-	nt.
D124	female	CP 1:128	++	++	-	-	-	-	-	nt.
D128	male	CP 1:512	+	++	-	-	++	+	++	-
D132	female	negative	++	++	-	-	+	+	-	-
D136	female	CP 1:128	+	++	-	-	+/-	-	-	_
D140	male	CP 1:256	++	++	-	-	+	+	-	-
D142	female	CP 1:512	++	++	-	_	+	+	+	-
D146	female	negative	++	++	-	-	++	+	+	-

CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant Chlamydia TSA protein. Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10⁵ PBMC with 1 x 10⁴ monocyte-derived dendritic cells pre-incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a ³H-thymidine pulse for the last 18h.

SI: Stimulation index

+/-:	SI ~	4
+:	SI>	4
++:	SI	10-30
+++:	SI >	30

In a first series of experiments, T-cell lines were generated from a healthy female individual (CT-10) with a history of genital exposure to *C. trachomatis* by stimulating T-cells with *C. trachomatis* LGV II elementary bodies as previously described. Although the study subject was exposed to *C. trachomatis*, she did not seroconvert and did not develop clinical symptoms, suggesting donor CT-10 may have developed a protective immune response against *C. trachomatis*. As shown in Fig. 10, a primary *Chlamydia*-specific T-cell line derived from donor CT-10 responded to *C. trachomatis*-SWIB, but not *C. pneumoniae*-SWIB recombinant proteins, confirming the exposure of CT-10 to *C. trachomatis*. Epitope mapping of the T-cell response to *C. trachomatis*-SWIB showed that this donor responded to the same epitope Ct-SWIB 52-67 (SEQ ID NO: 39) as T-cell line TCL-8, as shown in Fig. 11.

Additional T-cell lines were generated as described above for various C. trachomatis patients. A summary of the patients' clinical profile and proliferative responses to various C. trachomatis and C. pneumoniae elementary bodies and recombinant proteins are summarized in Table II.

Proliferative response of C. trachomatis patients						···				
atients	Clinical manifestation	IgG titer	CT	CP	CT Swib	CP Swib		CP S13	CT lpdA	CT TSA
CT-1	NGU	negative	+	+	-	-	++	++	++	+
CT-2	NGU	negative	++	++		-	+	+/-	-	-
CT-3	asymptomatic shed Eb Dx was HPV	Ct 1:512 Cp 1:1024 Cps 1:256	+	+	-	-	+	-	+	-
CT-4	asymptomatic shed Eb	Ct 1:1024	+	+	-	-	-	-	-	-
CT-5	BV	Ct 1:256 Cp 1:256	++	++	-	-	+	-	-	-
СТ-6	perinial rash discharge	Cp 1:1024	+	+	-	-	-	-	~	-
CT-7	BV genital ulcer	Ct 1:512 Cp 1:1024	+	+	-	-	+	+	+	-
CT-8	Not known	Not tested	++	++	-	-	-	-	-	-
CT-9	asymptomatic	Ct 1:128 Cp 1:128	+++	++	-	-	++	+	+	-
CT-10	Itch mild vulvar	negative	++	++	-	-	-	-	-	-
CT-11	BV, abnormal pap	Ct 1: 512	+++	+++	-	-	+++	+/-	++	+
CT-12	asymptomatic	Cp 1: 512	++	++	-	-	++	+	+	

NGU= Non-Gonococcal Urethritis; BV= Bacterial Vaginosis; CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant Chlamydia TSA protein
Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10⁵ PBMC with 1 x 10⁴ monocyte-derived dendritic cells pre-

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incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a ³H-thymidine pulse for the last 18 hours.

SI: Stimulation index

+/-: SI ~ 4 +: SI > 4 ++: SI 10-30 +++: SI 30

Using the panel of asymptomatic (as defined above) study subjects and C. trachomatis patients, as summarized in Tables I and II, a comprehensive study of the immune responses of PBMC derived from the two groups was conducted. Briefly, PBMCs from C. pneumoniae patients as well as from normal donors are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μ g/ml gentamicin. Purified polypeptides, a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and S13, as well as . C. trachomatis lpdA and TSA are added in duplicate at concentrations of 0.5 to 10 μ g/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μ l, 50 μ l of medium is removed from each well for determination of IFN- γ levels, as described below. The plates are then pulsed with 1 μ Ci/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

Proliferative responses to the recombinant *Chlamydiae* antigens demonstrated that the majority of asymptomatic donors and *C. trachomatis* patients recognized the *C. trachomatis* S13 antigen (8/12) and a majority of the *C. trachomatis* patients recognized the *C. pneumonia* S13 antigen (8/12), with 4/12 asymptomatic donors also recognizing the *C. pneumonia* S13 antigen. Also, six out of twelve of the *C. trachomatis* patients and four out of twelve of the asymptomatic donors gave a proliferative response to the lpdA antigen of *C. trachomatis*. These results demonstrate that the *C. trachomatis* and *C. pneumonia* S13 antigen, *C. trachomatis* Swib antigen and the *C. trachomatis* lpdA antigen are recognized by the asymptomatic donors, indicating these antigens were recognized during exposure to *Chlamydia* and an immune response elicited against them. This implies these antigens may

play a role in conferring protective immunity in a human host. In addition, the C. trachomatis and C. pneumonia S13 antigen is recognized equally well among the C. trachomatis patients, therefore indicating there may be epitopes shared between C. trachomatis and C. pneumonia in the S13 protein. Table III summarizes the results of these studies.

Table III.

Antigen	Normal Donors	C.t. Patients		
C.tSwib	3/12	0/12		
C.pSwib	0/12	0/12		
C.tS13	8/12	8/12		
C.pS13	4/12	8/12		
lpdA	4/12	6/12		
TSA	0/12	2/12		

A series of studies were initiated to determine the cellular immune response to short-term T-cell lines generated from asymptomatic donors and *C. trachomatis* patients. Cellular immune responses were measured by standard proliferation assays and IFN-γ, as described in Example 7. Specifically, the majority of the antigens were in the form of single *E. coli* clones expressing Chlamydial antigens, although some recombinant proteins were also used in the assays. The single *E. coli* clones were titered on 1 x 10⁴ monocyte-derived dendritic cells and after two hours, the culture was washed and 2.5 x 10⁴ T-cells were added. The assay using the recombinant proteins were performed as previously described. Proliferation was determined after four days with a standard ³H-thymidine pulse for the last 18 hours. Induction of IFN-γ was determined from culture supernatants harvested after four days using standard ELISA assays, as described above. The results show that all the *C. trachomatis* antigens tested, except for C.T. Swib, elicited a proliferative response from one or more different T-cell lines derived form *C. trachomatis* patients. In addition, proliferative responses were elicited from both the *C. trachomatis* patients and asymptomatic donors for

the following Chlamydia genes, CT622, groEL, pmpD, CT610 and rS13.

The 12G3-83 clone also contains sequences to CT734 and CT764 in addition to CT622, and therefore these gene sequence may also have immunoreactive epitopes. Similarly, clone 21G12-60 contains sequences to the hypothetical protein genes CT229 and CT228 in addition to CT875; and 15H2-76 also contains sequences from CT812 and CT088, as well as sharing homology to the sycE gene. Clone 11H3-61 also contains sequences sharing homology to the PGP6-D virulence protein.

Table IV.

Clone	C. t. Antigen	TCL from	TCL from	SEQ ID NO::
	(putative*)	Asymp. Donors	C. t. Patients	
1B1-66 (E. coli)	Swib	2/2	0/4	5
1B1-66 (protein)	Swib	2/2	0/4	5
12G3-83 (E. coli)	CT622*	2/2	4/4	57
22B3-53 (E. coli)	groEL	1/2	4/4	111
22B3-53 (protein)	groEL	1/2	4/4	111
15H2-76 (E. coli)	PmpD*	1/2	3/4	87
11H3-61 (E. coli)	rL1*	0/2	3/4	60
14H1-4 (E. coli)	TSA	0/2	3/4	56
14H1-4 (protein)	TSA	0/2	3/4	56
11G10-46 (E. coli)	CT610	1/2	1/4	62
10C10-17 (E. coli)	rS13	1/2	1/4	62
10C10-17 (protein)	rS13	1/2	1/4	62
21G12-60 (E. coli)	CT875*	0/2	2/4	110
11H4-32 (E. coli)	dnaK	0/2	2/4	59
21C7-8 (E. coli)	dnaK	0/2	2/4	115
17C10-31 (E. coli)	CT858	0/2	2/4	114

EXAMPLE 9

PROTECTION STUDIES USING CHLAMYDIA ANTIGENS

Protection studies were conducted in mice to determine whether immunization with chlamydial antigens can impact on the genital tract disease resulting from chlamydial inoculation. Two models were utilized; a model of intravaginal inoculation that uses a human isolate containing a strain of Chlamydia psittaci (MTW447), and a model of intrauterine inoculation that involves a human isolate identified as Chlamydia trachomatis, serovar F (strain NI1). Both strains induce inflammation in the upper genital tract, which resemble endometritis and salpingitis caused by Chlamydia trachomatis in women. In the first experiment, C3H mice (4 mice per group) were immunized three times with 100 µg of pcDNA-3 expression vector containing C. trachomatis SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5). Inoculations were at the base of the tail for systemic immunization. Two weeks after the last immunization, animals were progesterone treated and infected, either thru the vagina or by injection of the inoculum in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored (from + for very mild, to +++++ for very severe). Scores attributed to each single oviduct/ovary were summed and divided by the number of organs examined to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control-immunized animals receiving empty vector showed consistent inflammation with an ovary/oviduct mean inflammation score of 6.12, in contrast to 2.62 for the DNA-immunized group. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 8.37, versus 5.00 for the DNA-immunized group. Also, in the later model, vaccinated mice showed no signs of tubal occlusion while negative control vaccinated groups had inflammatory cells in the lumen of the oviduct

In a second experiment, C3H mice (4 mice per group) were immunized three times with 50 µg of pcDNA-3 expression vector containing *C. trachomatis* SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5) encapsulated in Poly Lactide co-Glycolide microspheres (PLG); immunizations were made

intra-peritoneally. Two weeks after the last immunization, animal were progesterone treated and infected by inoculation of *C. psittaci* in the vagina. Two weeks after infection, mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored as previously described. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean of inflammation for the group. Negative control-immunized animals receiving PLG-encapsulated empty vector showed consistent infammation with an ovary /oviduct mean inflammation score of 7.28, versus 5.71 for the PLG-encapsulated DNA immunized group. Inflammation in the peritoneum was 1.75 for the vaccinated group versus 3. 75 for the control.

In a third experiment, C3H mice (4 per group) were immunized three times with 10 µg of purified recombinant protein, either SWIB (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5, or S13 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12) mixed with Cholera Toxin (CT); the preparation was administred intranasally upon anaesthesia in a 20 uL volume. Two weeks after the last immunization, animal were progesterone treated and infected, either by vaginal inoculation of C. psittaci or by injection of C. trachomatis serovar F in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. The degree of inflammation was scored as described above. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control- immunized animals receiving cholera toxin alone showed an ovary /oviduct mean inflammation score of 4.25 (only 2 mice analyzed; 2 other died) versus 5.00 for the s13 plus cholera toxin-immunized group, and 1.00 for the SWIB plus cholera toxin. Untreated infected animals had an ovary/oviduct mean inflammation score of 7. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 7.37 versus 6.75 for the s13 plus cholera toxin-immunized group and 5.37 for the SWIB plus cholera toxin-immunized group. Untreated infected animals had an ovary /oviduct mean inflammation score of 8.

The three experiments described above suggest that SWIB-specific protection is obtainable. This protective effect is more marked in the model of homologous infection but is still present when in a heterologous challenge infection with *C. psittaci*.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

Claims

- 1. An isolated polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290; (b) sequences complementary to a sequence of (a); and (c) polynucleotide sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 2. The polypeptide of claim 1 wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 5, 26, 32, 65, 90, 92-98, 103-108, 121, 123, 125, 127, 129, 131, 133, 135, 137, 175-180, 189-196, 264 and 266.
- 3. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1 and 2.
- 4. A recombinant expression vector comprising a polynucleotide molecule according to claim 3.
 - 5. A host cell transformed with an expression vector according to claim 4.
- 6. The host cell of claim 5 wherein the host cell is selected from the group consisting of *E. coli*, yeast and mammalian cells.
- 7. A fusion protein comprising a polypeptide according to any one of claims 1 and 2.
- 8. A fusion protein according to claim 7, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell

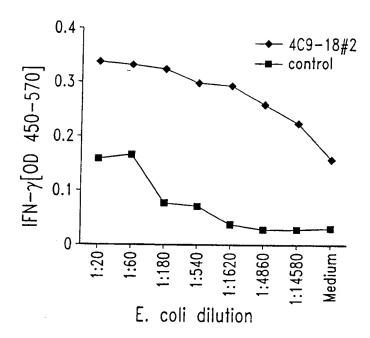


Fig. 1

2/10



Fig. 2

Chlamydia C17.8 Peptide Screen

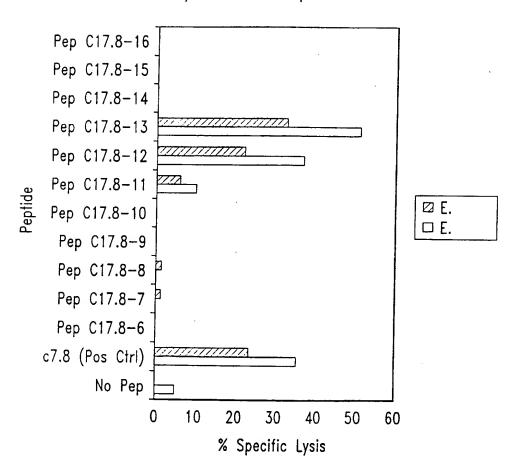
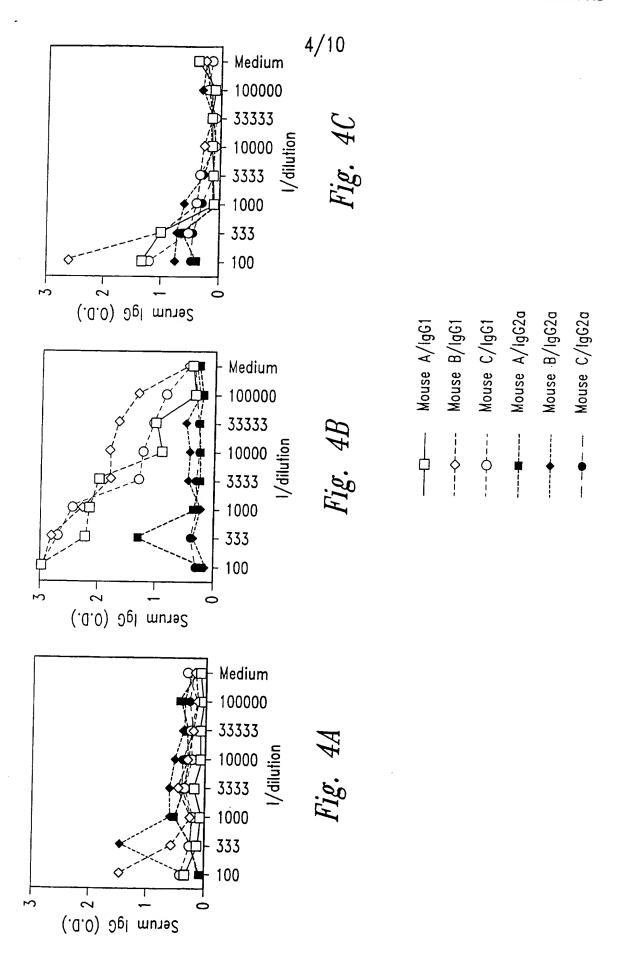
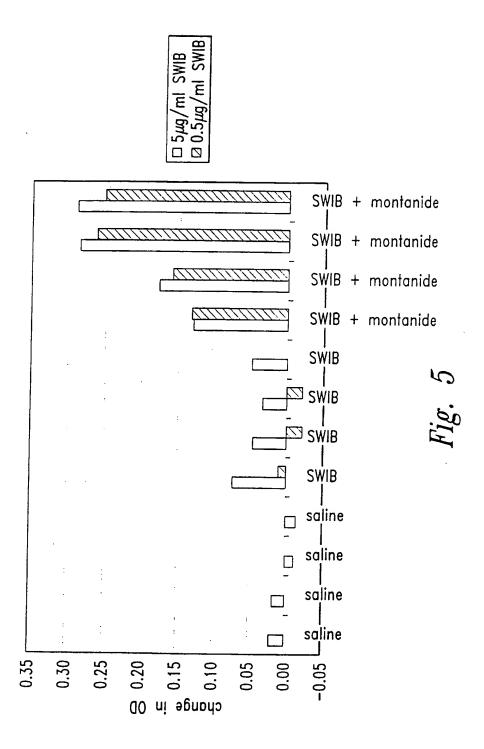


Fig. 3



Allanes --- --- ---- ----



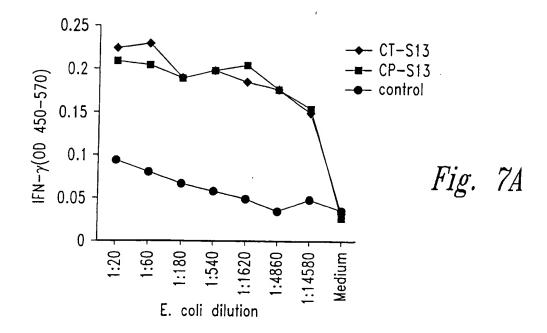
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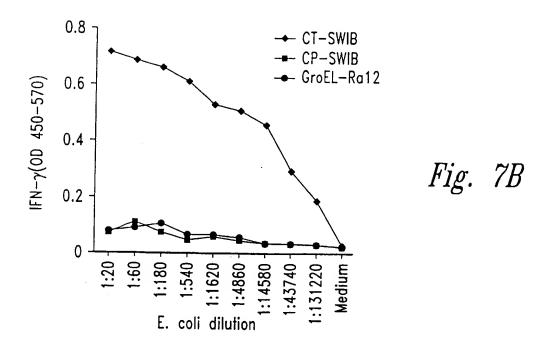
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Fig. 6





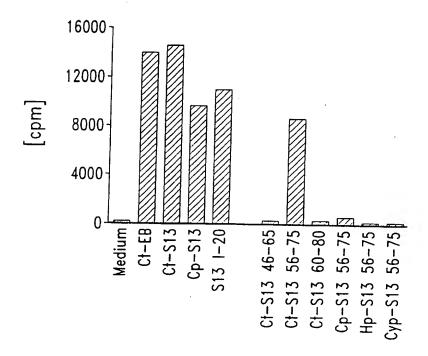
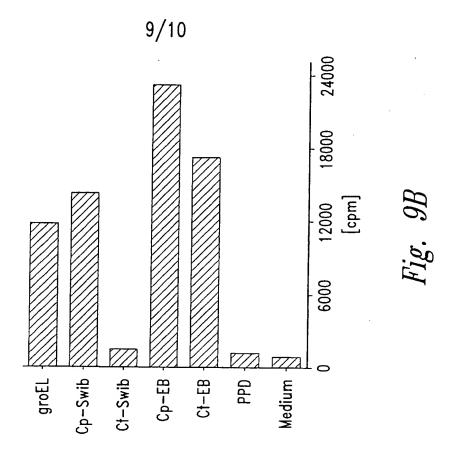
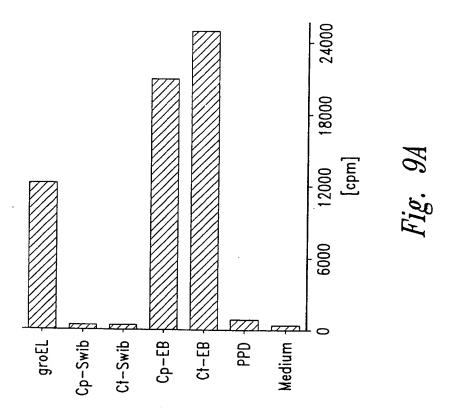


Fig. 8







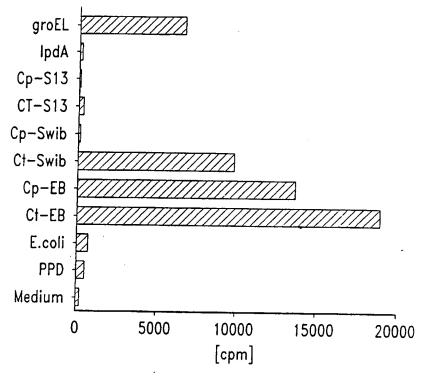
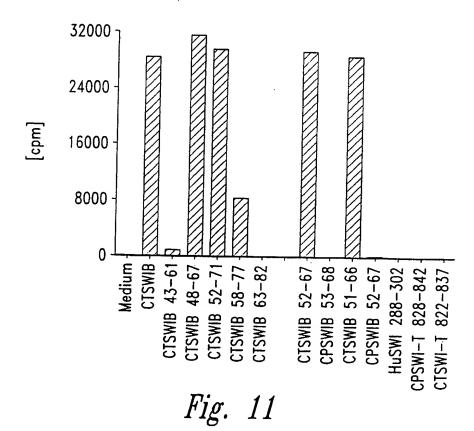


Fig. 10



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. 35

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	Gly	Gly	Val 20	_	Gly	Cys	Glu	Phe		Ser	Leu	Phe		15 Thr	Leu	
Gly	Ser	Glu 35		Ser	Val	Ile	Glu 40		Ser	Ser	Gln	Ile 45	30 Leu	Ala	Leu	
Asn	Asn 50		Asp	Ile	Ser	Lys 55		Met	Phe	Asp	Lys 60		Thr	Arg	Gln	
Gly 65	Leu	Arg	Phe	Val	Leu 70		Ala	Ser	Val	Ser	Asn	Ile	Glu	Asp		
	Asp	Arg	Val	Arg 85		Thr	Ile	Asn	Gly 90	Asn	Val	Glu	Glu		80 Asp	
Tyr	Val	Leu	Val		Ile	Gly	Arg	Arg			Thr	Glu		95 Ile	Gly	
Leu	Asp	Lys 115		Gly	Val	Ile	Cys 120		Glu	Arg	Gly	Val	110 Ile	Pro	Thr	
Asp	Ala 130		Met	Arg	Thr	Asn 135		Pro	Asn	Ile	Tyr 140	Ala	Ile	Gly	Asp	
Ile 145	Thr	Gly	Lys	Trp	Gln 150		Ala	His	Val	Ala 155	Ser	His	Gln	Gly		
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Ala	Val	Pro	Ser 180		Ile	Phe	Thr	Phe 185		Glu	Val	Ala	Ser 190	175 Val	Gly	
Leu	Ser	Pro 195		Ala	Ala	Gln	Gln 200	His	Leu	Leu	Leu	Arg 205	Leu	Leu	Phe	
Leu	Lys 210	Asn	Leu	Ile	Gln	Lys 215		Asn	Ser	Ser	His 220		Cys	Glu	Glυ	
Glu 225	Gly	Val	Trp	Lys	Thr 230						220					
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atra		00>			- ~+	~~~										
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cttt	ccaa	ac a	tatt	gtaa	a at	aa									_	264
		10> 3														
		11> { 12> }						-			•					
				nvđi.	a pn	eumo	niaa									
				,	~ pin	Cumo	11106									
Met (00> 2		٠	T	N	2 -						_			
Met :	JC1 (- 11 T	∟ys <i>I</i>	asn . 5	Lys A	ASN :	ser .		Phe 1 10	Met :	His 1	Pro 1		Asn : L5	Ile	
Ser 1		2	20					Gly : 25	Lys			-	Pro I	Arg T		
Glu :	Ile V			ys 7	Val :	Trp (Glu '	Tyr :	Ile 1	Lys :	Lys I	lis A	Asn (Cys (Gln	

45

```
Asp Gln Lys Asn Lys Arg Asn Ile Leu Pro Asp Ala Asn Leu Ala Lys
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 Val Phe Gly Ser Ser Asp Pro Ile Asp Met Phe Gln Met Thr Lys Ala
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                                25
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                            40
Thr Glu Glu Glu Val Gly Arg Leu Asn Ser Leu Leu Gln Ser Glu Tyr
Thr Val Glu Gly Asp Leu Arg Arg Val Gln Ser Asp Ile Lys Arg
                                        75
Leu Ile Ala Ile His Ser Tyr Arg Gly Gln Arg His Arg Leu Ser Leu
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5

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Leu Phe Val Asn Lys Met Leu Ala Gln Pro Phe Leu Ser Ser Gln Thr
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Lys Ala Asn Met Gly
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ttgcaaccgc acgcgattga atgatacgca agccatttcc atcatggaaa agaacccttg 180
gacaaaaata caaaggaggt tcactcctaa ccagaaaaag ggagagttag tttccatggg 240
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gttgttctag ctttggtacg agaaggtgat tctaagccct acgcgattag ttatggatac 420
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 aggaacattg aaactttatt aggaggaact gaaataggaa aattcacagt cacacccaaa 300
 agetetggga geatgttett agteteagea gatattattg cateaagaat ggaaggegge 360
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gccctttcta atggcaatga tattttagga ataacaaata cttctaatgt atcttttttg 480
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gaggtaatac ctcaaacaaa cgcttaaaca atttttattg gatttttctt ataggtttta 540
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aaaaatgatc gacaaggagc acgctaaatt tgtacatacc ccaaaatcaa tcagccatct 660
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 ggacgccgtt tgaatacaga aaatattggc ttggataaag ctggtgttat ttgtgatgaa 240
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35 40 45

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His Gly Ala Val Val Leu Gly Cys Ser Val Asp Asp Ile Glu Thr His 65 70 75 80

Ser Arg Trp Leu Thr Val Ala Arg Asp Ala Gly Gly Ile Glu Gly Thr 85 90 95

Glu Tyr Pro Leu Leu Ala Asp Pro Ser Phe Lys Ile Ser Glu Ala Phe 100 105 110

Gly Val Leu Asn Pro Glu Gly Ser Leu Ala Leu Arg Ala Thr Phe Leu 115 120 125

Ile Asp Lys His Gly Val Ile Arg His Ala Val Ile Asn Asp Leu Pro 130 135 140

Leu Gly Arg Ser Ile Asp Glu Glu Leu Arg Ile Leu Asp Ser Leu Ile 145 150 155 160

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Ile Lys Lys His Asn Cys Gln Asp Gln Lys Asn Lys Arg Asn Ile Leu 50 55 60

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Asp Ser Val Val Arg Ser Ile Arg Asp Gly Leu Asn Gly Leu Ile Arg

Ser Asn Lys Ile Thr Val Phe Ser Gly Arg Gly Ser Leu Ile Ser Ser 115 120 125

Thr Glu Val Lys Ile Leu Gly Glu Asn Pro Ser Val Ile Lys Ala His 130 135 140

Ser Ile Ile Leu Ala Thr Gly Ser Glu Pro Arg Ala Phe Pro Gly Ile 145 150 155 160

- Pro Phe Ser Ala Glu Ser Pro Arg Ile Leu Cys Ser Thr Gly Val Leu 165 170 175
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- Ile Gly Cys Glu Phe Ala Ser Leu Phe His Thr Leu Gly Ser Glu Val 195 200 205
- Ser Val Ile Glu Ala Ser Ser Gln Ile Leu Ala Leu Asn Asn Pro Asp 210 215 220
- Ile Ser Lys Thr Met Phe Asp Lys Phe Thr Arg Gln Gly Leu Arg Phe 225 230 235 240
- Val Leu Glu Ala Ser Val Ser Asn Ile Glu Asp Ile Gly Asp Arg Val 245 250 255
- Arg Leu Thr Ile Asn Gly Asn Val Glu Glu Tyr Asp Tyr Val Leu Val 260 265 270
- Ser Ile Gly Arg Arg Leu Asn Thr Glu Asn Ile Gly Leu Asp Lys Ala. 275 280 285
- Gly Val Ile Cys Asp Glu Arg Gly Val Ile Pro Thr Asp Ala Thr Met 290 295 300
- Arg Thr Asn Val Pro Asn Ile Tyr Ala Ile Gly Asp Ile Thr Gly Lys 305 310 315 320
- Trp Gln Leu Ala His Val Ala Ser His Gln Gly Ile Ile Ala Ala Arg 325 330 335
- Asn Ile Gly Gly His Lys Glu Glu Ile Asp Tyr Ser Ala Val Pro Ser 340 345 350
- Val Ile Phe Thr Phe Pro Glu Val Ala Ser Val Gly Leu Ser Pro Thr 355 360 365
- Ala Ala Gln Gln Lys Ile Pro Val Lys Val Thr Lys Phe Pro Phe 370 375 380
- Arg Ala Ile Gly Lys Ala Val Ala Met Gly Glu Ala Asp Gly Phe Ala 385 390 395 400
- Ala Ile Ile Ser His Glu Thr Thr Gln Gln Ile Leu Gly Ala Tyr Val 405 410 415
- Ile Gly Pro His Ala Ser Ser Leu Ile Ser Glu Ile Thr Leu Ala Val 420 425 430
- Arg Asn Glu Leu Thr Leu Pro Cys Ile Tyr Glu Thr Ile His Ala His 435 440 445
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<400> 91

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Glu Ala Arg Ala Ser Glu Leu Thr Glu Glu Glu Val Gly Arg Leu Asn 50 55 60

Ser Leu Leu Gln Ser Glu Tyr Thr Val Glu Gly Asp Leu Arg Arg 65 70 75 80

Val Gln Ser Asp Ile Lys Arg Leu Ile Ala Ile His Ser Tyr Arg Gly
85 90 95

Gln Arg His Arg Leu Ser Leu Pro Val Arg Gly Gln Arg Thr Lys Thr

Asn Ser Arg Thr Arg Lys Gly Lys Arg Lys Thr Val Ala Gly Lys Lys 115 120 125

Lys

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Arg Leu Val Asp Phe Glu Glu His Gly Ala Val Val Leu Gly Cys Ser 65 70 75 80

Val Asp Asp Ile Glu Thr His Ser Arg Trp Leu Thr Val Ala Arg Asp 85 90 95

Ala Gly Gly Ile Glu Gly Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser 100 105 110

Phe Lys Ile Ser Glu Ala Phe Gly Val Leu Asn Pro Glu Gly Ser Leu 115 120 125

Ala Leu Arg Ala Thr Phe Leu Ile Asp Lys His Gly Val Ile Arg His 130 135 140

Ala Val Ile Asn Asp Leu Pro Leu Gly Arg Ser Ile Asp Glu Glu Leu 145 150 155 160

Arg Ile Leu Asp Ser Leu Ile Phe Phe Glu Asn His Gly Met Val Cys 165 170 175

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Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr
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Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln
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105

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His Lys Arg Arg Ala Ala Ala Val Cys Ser Ile Ile Gly Gly Ile
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Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
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Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
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Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
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Val Gly Ala Gly Leu Ala Ile Ser Ala Glu Arg Ala Asp Cys Glu Ala
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Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Met Pro Gly
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Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
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Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
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 Thr Val Met Ala Leu Gly Asn Val Phe Asn Gly Ser Val Pro Ala Thr
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 Ile Gln Ser Ala Arg Ser Cys Leu Ala His Leu Arg Ala Ala Gly Lys
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Ser Gln Asp Asp Pro Leu Tyr Val Leu Gly Asn Ser Tyr Cys Trp Phe
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Val Ser Lys Leu His Ile Thr Asp Pro Lys Glu Ala Leu Phe Lys Glu
Lys Gly Asp Leu Ser Ile Gln Asn Phe Arg Phe Leu Ser Phe Thr Asp
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Gly	y Gly	/ Ala	a Il.	e Glr		Glr	Thr	Phe	e Sei		ı Se:	r Ar			s 1 Ser	
Pro	o Ile	Ser 195	? Phe		a Arg	Asn	Arg 200	Ala		Let	ı Ası				a Ile	
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40 ·

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9		Ile 915	SET	атХ	THE	υLU	7nr 920	ınr	ьeu	Leu	Ser	His 925	Gln	Glu	Thr

Trp Thr Thr Asp Ala Phe His Leu Ala Arg His Gly Val Val Val Arg 935 940 Gly Ser Met Tyr Ala Ser Leu Thr Ser Asn Ile Glu Val Tyr Gly His 950 955 Gly Arg Tyr Glu Tyr Arg Asp Ala Ser Arg Gly Tyr Gly Leu Ser Ala 965 970 Gly Ser Lys Val Xaa Phe 980 <210> 177 <211> 964 <212> PRT <213> Chlamydia <400> 177 Met Lys Lys Ala Phe Phe Phe Leu Ile Gly Asn Ser Leu Ser Gly Leu Ala Arg Glu Val Pro Ser Arg Ile Phe Leu Met Pro Asn Ser Val 25 Pro Asp Pro Thr Lys Glu Ser Leu Ser Asn Lys Ile Ser Leu Thr Gly 40 Asp Thr His Asn Leu Thr Asn Cys Tyr Leu Asp Asn Leu Arg Tyr Ile 55 Leu Ala Ile Leu Gln Lys Thr Pro Asn Glu Gly Ala Ala Val Thr Ile 70 75 Thr Asp Tyr Leu Ser Phe Phe Asp Thr Gln Lys Glu Gly Ile Tyr Phe Ala Lys Asn Leu Thr Pro Glu Ser Gly Gly Ala Ile Gly Tyr Ala Ser 100 105 Pro Asn Ser Pro Thr Val Glu Ile Arg Asp Thr Ile Gly Pro Val Ile 120 125 Phe Glu Asn Asn Thr Cys Cys Arg Leu Phe Thr Trp Arg Asn Pro Tyr 135 140 Ala Ala Asp Lys Ile Arg Glu Gly Gly Ala Ile His Ala Gln Asn Leu 150 155 Tyr Ile Asn His Asn His Asp Val Val Gly Phe Met Lys Asn Phe Ser 165 170 Tyr Val Gln Gly Gly Ala Ile Ser Thr Ala Asn Thr Phe Val Val Ser 180 185 Glu Asn Gln Ser Cys Phe Leu Phe Met Asp Asn Ile Cys Ile Gln Thr 200 205 Asn Thr Ala Gly Lys Gly Gly Ala Ile Tyr Ala Gly Thr Ser Asn Ser 220 Phe Glu Ser Asn Asn Cys Asp Leu Phe Phe Ile Asn Asn Ala Cys Cys 230 235 Ala Gly Gly Ala Ile Phe Ser Pro Ile Cys Ser Leu Thr Gly Asn Arg 245 250 Gly Asn Ile Val Phe Tyr Asn Asn Arg Cys Phe Lys Asn Val Glu Thr 265 Ala Ser Ser Glu Ala Ser Asp Gly Gly Ala Ile Lys Val Thr Thr Arg 280 Leu Asp Val Thr Gly Asn Arg Gly Arg Ile Phe Phe Ser Asp Asn Ile 295 Thr Lys Asn Tyr Gly Gly Ala Ile Tyr Ala Pro Val Val Thr Leu Val 305 310

Asp Asn Gly Pro Thr Tyr Phe Ile Asn Asn Ile Ala Asn Asn Lys Gly 325 330 Gly Ala Ile Tyr Ile Asp Gly Thr Ser Asn Ser Lys Ile Ser Ala Asp 340 345 Arg His Ala Ile Ile Phe Asn Glu Asn Ile Val Thr Asn Val Thr Asn 360 Ala Asn Gly Thr Ser Thr Ser Ala Asn Pro Pro Arg Arg Asn Ala Ile 375 380 Thr Val Ala Ser Ser Ser Gly Glu Ile Leu Leu Gly Ala Gly Ser Ser 390 395 Gln Asn Leu Ile Phe Tyr Asp Pro Ile Glu Val Ser Asn Ala Gly Val 410 Ser Val Ser Phe Asn Lys Glu Ala Asp Gln Thr Gly Ser Val Val Phe 425 Ser Gly Ala Thr Val Asn Ser Ala Asp Phe His Gln Arg Asn Leu Gln 440 Thr Lys Thr Pro Ala Pro Leu Thr Leu Ser Asn Gly Phe Leu Cys 1le 455 Glu Asp His Ala Gln Leu Thr Val Asn Arg Phe Thr Gln Thr Gly Gly 470 475 Val Val Ser Leu Gly Asn Gly Ala Val Leu Ser Cys Tyr Lys Asn Gly 490 Thr Gly Asp Ser Ala Ser Asn Ala Ser Ile Thr Leu Lys His Ile Gly 500 505 Leu Asn Leu Ser Ser Ile Leu Lys Ser Gly Ala Glu Ile Pro Leu Leu 515 520 Trp Val Glu Pro Thr Asn Asn Ser Asn Asn Tyr Thr Ala Asp Thr Ala 535 540 Ala Thr Phe Ser Leu Ser Asp Val Lys Leu Ser Leu Ile Asp Asp Tyr 550 555 Gly Asn Ser Pro Tyr Glu Ser Thr Asp Leu Thr His Ala Leu Ser Ser 565 570 Gln Pro Met Leu Ser Ile Ser Glu Ala Ser Asp Asn Gln Leu Gln Ser 580 585 Glu Asn Ile Asp Phe Ser Gly Leu Asn Val Pro His Tyr Gly Trp Gln 600 Gly Leu Trp Thr Trp Gly Trp Ala Lys Thr Gln Asp Pro Glu Pro Ala 615 Ser Ser Ala Thr Ile Thr Asp Pro Gln Lys Ala Asn Arg Phe His Arg 630 635 Thr Leu Leu Thr Trp Leu Pro Ala Gly Tyr Val Pro Ser Pro Lys 650 His Arg Ser Pro Leu Ile Ala Asn Thr Leu Trp Gly Asn Met Leu Leu 665 Ala Thr Glu Ser Leu Lys Asn Ser Ala Glu Leu Thr Pro Ser Gly His 680 Pro Phe Trp Gly Ile Thr Gly Gly Gly Leu Gly Met Met Val Tyr Gln 695 Asp Pro Arg Glu Asn His Pro Gly Phe His Met Arg Ser Ser Gly Tyr 710 715 Ser Ala Gly Met Ile Ala Gly Gln Thr His Thr Phe Ser Leu Lys Phe 725 730 Ser Gln Thr Tyr Thr Lys Leu Asn Glu Arg Tyr Ala Lys Asn Asn Val 745 Ser Ser Lys Asn Tyr Ser Cys Gln Gly Glu Met Leu Phe Ser Leu Gln

765

760

Glu Gly Phe Leu Leu Thr Lys Leu Val Gly Leu Tyr Ser Tyr Gly Asp 775 His Asn Cys His His Phe Tyr Thr Gln Gly Glu Asn Leu Thr Ser Gln 790 795 Gly Thr Phe Arg Ser Gln Thr Met Gly Gly Ala Val Phe Phe Asp Leu 810 Pro Met Lys Pro Phe Gly Ser Thr His Ile Leu Thr Ala Pro Phe Leu 825 Gly Ala Leu Gly Ile Tyr Ser Ser Leu Ser His Phe Thr Glu Val Gly 840 Ala Tyr Pro Arg Ser Phe Ser Thr Lys Thr Pro Leu Ile Asn Val Leu 855 Val Pro Ile Gly Val Lys Gly Ser Phe Met Asn Ala Thr His Arg Pro 870 875 Gln Ala Trp Thr Val Glu Leu Ala Tyr Gln Pro Val Leu Tyr Arg Gln 885 8.90 Glu Pro Gly Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe 905 Gly Ser Gly Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser 920 Gln Gln Thr Gln Pro Leu Ser Trp Leu Thr Leu His Phe Gln Tyr His 940 935 Gly Phe Tyr Ser Ser Ser Thr Phe Cys Asn Tyr Leu Asn Gly Glu Ile 950 955 Ala Leu Arg Phe <210> 178 <211> 1530 <212> PRT <213> Chlamydia Met Ser Ser Glu Lys Asp Ile Lys Ser Thr Cys Ser Lys Phe Ser Leu 5 Ser Val Val Ala Ala Ile Leu Ala Ser Val Ser Gly Leu Ala Ser Cys 25 Val Asp Leu His Ala Gly Gly Gln Ser Val Asn Glu Leu Val Tyr Val 40 Gly Pro Gln Ala Val Leu Leu Asp Gln Ile Arg Asp Leu Phe Val 55 Gly Ser Lys Asp Ser Gln Ala Glu Gly Gln Tyr Arg Leu Ile Val Gly 75 Asp Pro Ser Ser Phe Gln Glu Lys Asp Ala Asp Thr Leu Pro Gly Lys 85 90 Val Glu Gln Ser Thr Leu Phe Ser Val Thr Asn Pro Val Val Phe Gln 105 Gly Val Asp Gln Gln Asp Gln Val Ser Ser Gln Gly Leu Ile Cys Ser 120 Phe Thr Ser Ser Asn Leu Asp Ser Pro Arg Asp Gly Glu Ser Phe Leu 135 140 Gly Ile Ala Phe Val Gly Asp Ser Ser Lys Ala Gly Ile Thr Leu Thr 150 155 Asp Val Lys Ala Ser Leu Ser Gly Ala Ala Leu Tyr Ser Thr Glu Asp

Leu Ile Phe Glu Lys Ile Lys Gly Gly Leu Glu Phe Ala Ser Cys Ser Ser Leu Glu Gln Gly Gly Ala Cys Ala Ala Gln Ser Ile Leu Ile His Asp Cys Gln Gly Leu Gln Val Lys His Cys Thr Thr Ala Val Asn Ala Glu Gly Ser Ser Ala Asn Asp His Leu Gly Phe Gly Gly Gly Ala Phe Phe Val Thr Gly Ser Leu Ser Gly Glu Lys Ser Leu Tyr Met Pro Ala Gly Asp Met Val Val Ala Asn Cys Asp Gly Ala Ile Ser Phe Glu Gly Asn Ser Ala Asn Phe Ala Asn Gly Gly Ala Ile Ala Ala Ser Gly Lys Val Leu Phe Val Ala Asn Asp Lys Lys Thr Ser Phe Ile Glu Asn Arg Ala Leu Ser Gly Gly Ala Ile Ala Ala Ser Ser Asp Ile Ala Phe Gln Asn Cys Ala Glu Leu Val Phe Lys Gly Asn Cys Ala Ile Gly Thr Glu Asp Lys Gly Ser Leu Gly Gly Gly Ala Ile Ser Ser Leu Gly Thr Val Leu Leu Gln Gly Asn His Gly Ile Thr Cys Asp Lys Asn Glu Ser Ala Ser Gln Gly Gly Ala Ile Phe Gly Lys Asn Cys Gln Ile Ser Asp Asn Glu Gly Pro Val Val Phe Arg Asp Ser Thr Ala Cys Leu Gly Gly Ala Ile Ala Ala Gln Glu Ile Val Ser Ile Gln Asn Asn Gln Ala Gly Ile Ser Phe Glu Gly Gly Lys Ala Ser Phe Gly Gly Ile Ala Cys Gly Ser Phe Ser Ser Ala Gly Gly Ala Ser Val Leu Gly Thr Ile Asp Ile Ser Lys Asn Leu Gly Ala Ile Ser Phe Ser Arg Thr Leu Cys Thr Thr Ser Asp Leu Gly Gln Met Glu Tyr Gln Gly Gly Ala Leu Phe Gly Glu Asn Ile Ser Leu Ser Glu Asn Ala Gly Val Leu Thr Phe Lys Asp Asn Ile Val Lys Thr Phe Ala Ser Asn Gly Lys Ile Leu Gly Gly Gly Ala Ile Leu Ala Thr Gly Lys Val Glu Ile Thr Asn Asn Ser Gly Gly Ile Ser Phe Thr Gly Asn Ala Arg Ala Pro Gln Ala Leu Pro Thr Gln Glu Glu Phe Pro Leu Phe Ser Lys Lys Glu Gly Arg Pro Leu Ser Ser Gly Tyr Ser Gly Gly Gly Ala Ile Leu Gly Arg Glu Val Ala Ile Leu His Asn Ala Ala Val Val Phe Glu Gln Asn Arg Leu Gln Cys Ser Glu Glu Glu Ala Thr Leu Leu Gly Cys Cys Gly Gly Ala Val His

	61)				615	5				620	0			e Gly
625	5				630)				635	5				Ser 640
Lys	s Thi	r Val	l Glr	Let 645	ı Ala	Gly	Ası	n Gly	y Sea 650		l Asp	Phe	e Sei	Arg	g Asn
Ile	e Ala	s Sei	Leu 660	Gly	/ Gly	Gly	Ala	Lei 665		n Ala	a Sei	Glu	Gly 670	/ Ası	n Cys
		675	5				680)				685	5	_	/ Arg
	690) .				695				•	700)			Ser
Gly 705	r Asr	Lys	Gly	Arc	y Val 710	Glu	Phe	: Lys	s Asp	Asn 715		Ala	Thr	Arc	Leu
		Gl	Glu	Thr 725	. Val		Lys	. Val	1 Glu 730	Glu		G].u	Pro		720 Pro
Glu	Gln	Lys	Asp 740	Asn		Glu	Leu	Ser	Pne		Gly	Ser	Val		Gln
Ser	Phe	1le 755	Thr	Ala	Ala	Asn	Gln 760	Ala		Phe	Ala	Ser 765	Glu	Asp	Gly
	770				Ser	775					780	Leu	Ala		
785					Gly 790					795	Arg	Val			800
				805					310					815	Tyr
			820		Thr			825					830	Leu	Asp
		835			Val		840					845			
	850				Ser	855					860				
865					Thr 870					875					880
				885	Asn				890					895	
			900		Asn			905					910		
		3 T 2			Ser		920					925			
	930				Glu	935					940				
243					Phe 950					955					960
				965	Glu				970					975	
			980		Ile			985					990		
		995			Gln		1000	l .				1005			
	TOTO					1015					1020				
Leu 1025	Ala	Ala	Gly	Ser	Lys 1030	Leu	Lys	Ile	Leu	Asp 1035		Gly	Thr		Val 1040
		His	Ala	Ile	Ser :	Lys	Pro	Glu	Ala	Glu	Ile	Glu	Ser	Ser	Ser

				104					105					105	
Glu	Pro	Glu	Gly 106		His	Ser	Leu	Trp		Ala	Lys	Asn	Ala 107		1 Thr
Thr	Val	Pro 107		Val	Asp	Ile	His 108		Il€	Ser	· Val	. Asp		Ala	ser Ser
Phe	Ser 109		Ser	Gln	Gln	Glu 109	Gly		Val	. Glu	Ala	Pro		Val	Ile
Val	Pro	Glv	Glv	Ser	Tvr			Ser	์ Glv	r Glu			Τ.Δ.	C1 v	Leu
110		•	-		111		5		011	111		71011	. 1100	GIU	1120
Val	Asn	Thr	Thr	Gly			Tvr	Glu	Asn	His	Ala	Leu	Len	Tays	Asn
				112		•	•		113			_,	~	113	
Glu	Ala	Lys	Val	Pro	Leu	Met	Ser	Phe			Ser	Ser	Asp	Glu	Ala
			114	0					5				115		11.14
Ser	Ala	Glu	Ile	Ser	Asn	Leu	Ser				Leu	Gln	Ile	His	Val
		115	5				116	C				116	5		
Ala	Thr	Pro	Glu	Ile	Glu	Glu	Asp	Thr	Tyr	Gly	His	Met	Gly	Asp	Trp
	117	0				117	5				118	0			
Ser	Glu	Ala	Lуз	Ile	Gln	Asp	Gly	Thr	Leu	Val	Ile	Asn	Trp	Asn	Pro
118	5				119	0				119	5				1200
Thr	Gly	Tyr	Arg	Leu	Asp	Pro	Gln	Lys	Ala	Gly	Ala	Leu	Val	Phe	Asn
	_			120					121	0				121	5
Ala	Leu	Trp	Glu	Glu	Gly	Ala	Val			Ala	Leu	Lys	Asn	Ala	Arg
D.L	27-	** '	1220					122					123	0	
Рпе	Ala	HIS	Asn	Leu	Thr	Ala			Met	Glu	Phe	Asp		Ser	Thr
705	Wa I	1235		Db.		D1	124		_,			124			
ASII	1250		GIY	Pne	Ala			Giy	Phe	Arg		Leu	Ser	Ala	Glu
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		Val	Δen	Tla			Mot	C1	7	127		Leu	a1 .		1280
	019	·uı	лър	1285	G.111	шец	MEL	Giu	129		vai	Leu.	GIY		
Glv	Ala	Ala	Phe			Lvs	Met	Δen			Tvc	Phe	λαν	129	יי ייזיי
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Val	Ser	Arg			Val	Val	Glv			Tyr	Thr	Gly	Dhe	i Len	בות
		1315	5	•			1320)		-] -		1325		LCu	AIU
Gly	Ser	Trp	Phe	Phe	Lys	Gly	Gln	Tyr	Ser	Leu	Glv	Glu	Thr	Gln	Asn
	1330)				1339	5				1340)			
Asp	Met	Lys	Thr	Arg	Tyr	Gly	Val	Leu	Gly	Glu	Ser	Ser	Ala	Ser	Trp
1345	5				1350)				1355	5				1360
Thr	Ser	Arg	Gly	Val	Leu	Ala	Asp	Ala	Leu	Va].	Glu	Tyr	Arg	Ser	Leu
				1365					1370)				1379	5
Val	Gly	Pro	Val	Arg	Pro	Thr	Phe	Tyr	Ala	Leu	His	Phe	Asn	Pro	Tyr
			1380					1385	5 .				1390)	
Val	Glu	Val	Ser	Tyr	Ala	Ser	Met	Lys	Phe	Pro	Gly	Phe	Thr	Glu	Gln
		1395					1400)				1405	,		
зіу	Arg	Glu	Ala	Arg	Ser	Phe	Glu	Asp	Ala	Ser	Leu	Thr	Asn	Ile	Thr
- 1 -	1410				_	1415					1420)			
TTE	Pro	Leu	GIY	Met	Lys	Phe	Glu	Leu	Ala			Lys	Gly	Gln	Phe
1425		17.57	n -		1430			_		1435	;				1440
ber	GIU	val	Asn	ser	ьeu	GŢĀ	Ile	Ser	Tyr	Ala	Trp	Glu	Ala	Tyr	Arg
(Arc	17 n 1	C1		1445		37- 3	~ 1	_	1450					1455	;
чХS	val	GIU	G1y 1460	σιλ	нта	vai	GID			Glu	Ala	Gly			Trp
3111	Glv			Me+	Δαη	T.011	Dro	1465		C 1	T	B	1470	. 1 -	7
	1	1475			طوده	⊿ ∈u	1480		GIU	GIU		Arg		AIA	тел
		, -					7 Z O O					1485			

Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe Ser Thr Val Leu Gly Leu
1490 1495 1500

Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr Asp Ser Lys Leu Gly Tyr
1505 1510 1515 1520

Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe
1525 1530

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Ile	e Thi	r Gl	y Ile 340	e Thi	c Gly	Th	r Ile	e Asp 345		e Vai	l Ser	c Ası	n Ile 350		a Thr
		35	5				360)				365	Cys	5 Thr	Asn
	370)				375	5				380)			, Gly
385	5				390)				395	5				Glu 400
				405	5				410)				415	Asn
			420)				425	•				430)	Leu
		435	5				440)				445	,		Ser
	450)				455	,				460				Thr
465					470					475					Glu 480
				485					490					495	Glu
			500					505					510		Gln
		515					520					525	Ile		
	530					535					540		Lys		
545					550					555			Asn		560
				565					570				Cys	575	
			580					585					His 590		
		595					600					605	Val		
	9T0					615					620		۷al		
625					630					635					640
				645					650				Thr	655	
			660					665					Thr 670		
		6/5					680					685	Ser		
	690					695					700		Gln		
705					710					715			Asn		720
				725					730				Asp	735	
			740					745					Asp 750		
		755					760					765	Ser		
A1 a	Cys	Leu	нтġ	тÀ2	ser	ıyr	Ala	Ala	Ser	Thr	Asp	Ser	Ser	Pro	Val

	770	0				775	;				780)			
Ser 785	Ası	n Sei	Ser	Gly	7 Ser	Asp		. Thr	Ala	Ser 795	Ser) Asr	Pro	Asp 800
Ser	Sei	Sei	Ser	Gly 805	Asp	Ser	Ala	Gly	Asp 810	Ser		Gly	/ Pro	Thr 815	Glu
			820)				825	;				830	Gly	Gly
		835	5				840					845	Gly	Gln	Gly
	850)			Lys	855					860				
865					Leu 870					875					880
				885					890					895	
			900		Ser			905					910		
		915			Thr		920					925	,		
	930				Ala	935					940				
945	Pne	GIY	GIY	Ата	Ile 950	GIY	Ala	Thr	Ser	Ala 955	Val	Ser	Leu	Ser	Gly 960
				965	Glu				970	Leu				975	Gly
			980		Gln			985					990	Ser	_
		995			Lys		1000)				1009	Thr		
	101	U			Ile	1015	5				1020)			
1025)				Gly 1030)				1035	5				1040
				1045					1050)				1055	Pro
			1060)	Thr			1065	5				1070	١	
		1075)		Ala		1080)				1085	5		
	1090)				1095	,				1100				
Asn 1105	Ile	Cys	Phe	Arg	Asn 1110	Asn	Glu	Tyr	Arg			Ser	Ser	Asp	
		Ser	Thr	Phe 1125	Cys	Ser	Ile	Ala	Gly 1130	1115 Asp	Val	Lys	Leu		
Gln	Ala	Ala	Lys 1140	Gly	Lys	Thr	Ile	Ser 1145	Phe	Phe	Asp	Ala	Ile 1150		Thr
Ser	Thr	Lys 1155	Lys	Thr	Gly '	Thr	Gln 1160	Ala	Thr	Ala		Asp 1165	Thr	Leu	Asp
	TIV	,				Ser 1175	Glu	Thr			Ser .	Ala	Phe		
1190					Ser (Glu :	Leu			Asn 1195	Lys				1200
Gln	Asn	Val	Val	Leu 1205	His S	Ser (Gly		Leu 1210	Val :	Leu :	Lys		Asn ' 1215	Thr

GIU	Let	l His	Val 122	. Ile 20	e Sei	r Phe	e Glu	ı Glı 122		s Glu	ı Gly	y Sei	r Sei 123		ı Val
Met	Thr	Pro	Gly	. Sei	r Val	l Lei	ı Ser	Ası	ı Glı	n Thi	r Va	: [ג	a Acr	o Gla	/ Ala
		123	5				124	10				124	15		
Leu	vai 125	. Ile 50	Asn	Asr	n Met	Th:		e Asr		ı Ser	Ser 126		l Gli	ı Lys	s Asn
Glv	Ile	Ala	Glu	GIV	, Der					D 200			. 7	. . .	lle
126	5		010		127	. II.		. 1111	. FIC			т тес	ı Arç	1 TTE	
		Thr	Th~	Cov			. 01-	- 01		127					1280
				128	35				129	90				129	Glu 5
Ser	Asn	Gln	Asn 130		Asp) Asp	Thr	Lys 130		ı Glm	Asr	Asr	n Asn 131		Ala
Ser	Asn	Gln 131	Gly	Glu	Ser	Ala		_			Ser		Ala	Val	Ala
λ l ¬	አገ።			0		an)	132					132			
	133	0				133	5				134	0			Ala
Thr	Pro	Thr	Thr	Thr	Pro	Thr	Ala	Thr	Thr	Thr	Thr	Ser	Asn	Gln	Val
134	5				135					135					1360
Ile	Leu	Gly	Gly	Glu	Ile	Lys	Leu	Ile	Asp	Pro	Asn	Glv	Thr	Phe	Phe
				136	5	•				0		0-7		137	
Gln	Asn	Pro	Ala	Leu	Ara	Ser	Agn	Gln	Gln	Tla	Sar	LOU	T 011	137	Leu
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		Gln	Trn	አነ።			D	.		143	5				1440
ıyı	Arg	GIII	тър	ATA	LIÀL	vaı	Pro	Arg	Asp	Asn	His	Phe	Tyr	Ala	Asn
502	т1.	T	01	144			_		145	0				145.	5
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¥ _		~	1460					146					147	0	
ьeu	Asn	Asp	Lys	Met	Asn	Leu			Phe	Asp	Glu	Val	Ser	Tyr	Asn
_		1475					1480					148	5		
Asn	Leu	Trp	Ile	Ser	Gly	Leu	Gly	Thr	Met	Leu	Ser	Gln	Val	Gly	Thr
	1490)				149	5				150	0			
Pro	Thr	Ser	Glu	Glu	Phe	Thr	Tyr	Tyr	Ser	Arg	Glv	Ala	Ser	Val	Ala
1505)				1510)				1519	5				1520
Leu	Asp	Ala	Lys	Pro	Ala	His	qaA	Val	Ile	Val	Glv	Ala	Δla	Phe	Ser
				1525	5		•		1530	0	1			1535	
Lys	Met	Ile	Gly	Lys	Thr	Lvs	Ser	Leu	Lvs	Arg	Glu	Nen	λαn	Ture.	, Th∞
			1540) 1		2 -		1545	:	y	GIU	Wall			1111
His	Lvs	Glv			Tur	Car	ጥሙ	C12	י אות	Ser	17-1		1550) ~1	_
		1555					1560)				1565	5		
	1570)				1575	5			Glu	1580)			
Leu	Leu	Gln	Gly	Val	Ile	Ser	Tvr	Glv	Tvr	Ile	Lvs	His	Asn	Thr	Val
1585					1590)	4 -	2	- ,	1595		****	1101	T * * * T	
Thr	His	Tyr	Pro	Thr	Ile		Glu	Arg		Gln	Gly	Glu	Trp	Glu	1600 Asp
ĭ 0	C1	m		1605		_	_		1610)				1615	i
⊔eu	GTÅ	Trp	ьeu	Thr	Ala	Leu	Arg	Val	Ser	Ser	Val	Leu	Arg	Thr	Pro
			1620					1625	,				1630		
Ala	Gln	Gly .	Asp	Thr	Lys	Arg	Ile	Thr	Val	Tyr	Gly	Glu	Leu	Glu	Tyr
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Ser	Ser	Ile .	Arg	Gln	Lvs	Gln	Phe	Thr	Glu	Thr	Glu	Tur	λcn	Dro	λνα

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Ser Tyr Ala Asp His Glu Ser Phe Thr Glu Glu Gly Asp Gln Ala Arg 885 890 Ala Phe Lys Ser Gly His Leu Leu Asn Leu Ser Val Pro Val Gly Val 905 Lys Phe Asp Arg Cys Ser Ser Thr His Pro Asn Lys Tyr Ser Phe Met 920 Ala Ala Tyr Ile Cys Asp Ala Tyr Arg Thr Ile Ser Gly Thr Glu Thr 935 Thr Leu Leu Ser His Gln Glu Thr Trp Thr Thr Asp Ala Phe His Leu 950 Ala Arg His Gly Val Val Val Arg Gly Ser Met Tyr Ala Ser Leu Thr 965 .970 Ser Asn Ile Glu Val Tyr Gly His Gly Arg Tyr Glu Tyr Arg Asp Ala 985 Ser Arg Gly Tyr Gly Leu Ser Ala Gly Ser Lys Val Arg Phe 1000 <210> 191 <211> 977 <212> PRT <213> Chlamydia <400> 191 Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Ser Ser Leu Val Pro Ser Ser Asp Pro His His His His His Gly Leu Ala Arg 25 Glu Val Pro Ser Arg Ile Phe Leu Met Pro Asn Ser Val Pro Asp Pro Thr Lys Glu Ser Leu Ser Asn Lys Ile Ser Leu Thr Gly Asp Thr His 60 Asn Leu Thr Asn Cys Tyr Leu Asp Asn Leu Arg Tyr Ile Leu Ala Ile 70 75 Leu Gln Lys Thr Pro Asn Glu Gly Ala Ala Val Thr Ile Thr Asp Tyr Leu Ser Phe Phe Asp Thr Gln Lys Glu Gly Ile Tyr Phe Ala Lys Asn 105 Leu Thr Pro Glu Ser Gly Gly Ala Ile Gly Tyr Ala Ser Pro Asn Ser 120 125 Pro Thr Val Glu Ile Arg Asp Thr Ile Gly Pro Val Ile Phe Glu Asn 135 Asn Thr Cys Cys Arg Leu Phe Thr Trp Arg Asn Pro Tyr Ala Ala Asp 150 155 Lys Ile Arg Glu Gly Gly Ala Ile His Ala Gln Asn Leu Tyr Ile Asn 170 His Asn His Asp Val Val Gly Phe Met Lys Asn Phe Ser Tyr Val Gln 185 Gly Gly Ala Ile Ser Thr Ala Asn Thr Phe Val Val Ser Glu Asn Gln 200 Ser Cys Phe Leu Phe Met Asp Asn Ile Cys Ile Gln Thr Asn Thr Ala 215 Gly Lys Gly Gly Ala Ile Tyr Ala Gly Thr Ser Asn Ser Phe Glu Ser 230 235 Asn Asn Cys Asp Leu Phe Phe Ile Asn Asn Ala Cys Cys Ala Gly Gly 245 250

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	290					295	,				300				Val
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Tyr	Gly	Gly	Ala	1le 325		Ala	Pro	Val	Val	Thr		Val	Asp	Asn 335	Gly
Pro	Thr	Tyr	Phe		Asn	Asn	Ile	Ala 345		Asn	Lys	Gly	Gly 350	Ala	Ile
Tyr	Ile	Asp 355	Gly	Thr	Ser	Asn	Ser 360		Ile	Ser	Ala	Asp	Arg	His	Ala
Ile	Ile 370	Phe	Asn	Glu	Asn	Ile 375		Thr	Asn	Val	Thr			Asn	Gly
Thr 385	Ser	Thr	Ser	Ala	Asn 390		Pro	Arg	Arg	Asn 395		Ile	Thr	Val	Ala 400
Ser	Ser	Ser	Gly	Glu 405	Ile	Leu	Leu	Gly	Ala 410	Gly		Ser	Gln	Asn 415	Leu
Ile	Phe	Tyr	Asp 420	Pro	Ile	Glu	Val	Ser 425	Asn	Ala	Gly	Val	Ser 430	Val	Ser
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Thr	Val 450	Asn	Ser	Ala	Asp	Phe 455	His	Gln	Arg	Asn	Leu 460	Gln	Thr	Lys	Thr
Pro 465	Ala	Pro	Leu	Thr	Leu 470	Ser	Asn	Gly	Phe	Leu 475	Сув	Ile	Glu	Asp	His 480
Ala	Gln	Leu	Thr	Val 485	Asn	Arg	Phe	Thr	Gln 490		Gly	Gly	Val	Val 495	
Leu	Gly	Asn	Gly 500	Ala	Val	Leu	Ser	Cys 505	Туr	Lys	Asn	Gly	Thr 510		Asp
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	530					535	Ala				540				
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Pro	Tyr	Glu	Ser 580	Thr	Asp	Leu	Thr	His 585	Ala	Leu	Ser	Ser	Gln 590	Pro	Met
Leu	Ser	Ile 595	Ser	Glu	Ala	Ser	Asp 600	Asn	Gln	Leu	Gln	Ser 605	Glu	Asn	Ile
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Thr 625	Trp	Gly	Trp	Ala	Lys 630	Thr	Gln	Asp	Pro	Glu 635	Pro	Ala	Ser	Ser	Ala 640
Thr	Ile	Thr	Asp	Pro 645		Lys	Ala		Arg 650		His	Arg			Leu
Leu	Thr	Trp	Leu 660		Ala	Gly				Ser	Pro		His 670	655 Arg	Ser
Pro	Leu	Ile 675		Asn	Thr	Leu	Trp 680		Asn	Met		Leu 685	Ala	Thr	Glu
Ser			Asn	Ser	Ala	Glu		Thr	Pro	Ser			Pro	Phe	Trp

690 695 700 Gly Ile Thr Gly Gly Gly Leu Gly Met Met Val Tyr Gln Asp Pro Arg 710 715 Glu Asn His Pro Gly Phe His Met Arg Ser Ser Gly Tyr Ser Ala Gly 725 730 Met Ile Ala Gly Gln Thr His Thr Phe Ser Leu Lys Phe Ser Gln Thr 745 Tyr Thr Lys Leu Asn Glu Arg Tyr Ala Lys Asn Asn Val Ser Ser Lys 760 Asn Tyr Ser Cys Gln Gly Glu Met Leu Phe Ser Leu Gln Glu Gly Phe 775 Leu Leu Thr Lys Leu Val Gly Leu Tyr Ser Tyr Gly Asp His Asn Cys 790 795 His His Phe Tyr Thr Gln Gly Glu Asn Leu Thr Ser Gln Gly Thr Phe 805 810 Arg Ser Gln Thr Met Gly Gly Ala Val Phe Phe Asp Leu Pro Met Lys 820 825 Pro Phe Gly Ser Thr His Ile Leu Thr Ala Pro Phe Leu Gly Ala Leu 840 Gly Ile Tyr Ser Ser Leu Ser His Phe Thr Glu Val Gly Ala Tyr Pro 855 Arg Ser Phe Ser Thr Lys Thr Pro Leu Ile Asn Val Leu Val Pro Ile 870 875 Gly Val Lys Gly Ser Phe Met Asn Ala Thr His Arg Pro Gln Ala Trp 885 890 Thr Val Glu Leu Ala Tyr Gln Pro Val Leu Tyr Arg Gln Glu Pro Gly 905 Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe Gly Ser Gly 920 Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser Gln Gln Thr 935 Gln Pro Leu Ser Trp Leu Thr Leu His Phe Gln Tyr His Gly Phe Tyr 950 955 Ser Ser Ser Thr Phe Cys Asn Tyr Leu Asn Gly Glu Ile Ala Leu Arg 965 970 Phe

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<211> 848

<212> PRT

<213> Chlamydia

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145	,				150)				155	5		ı Ile		160
				165	5				170)			s Arg	175	,
			180)				185	i				190	1	
		195	5				200	1				205			_
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	450					455					460		Ser		
465					470					475			Ser		480
				485					490				Asp	495	
			500					505					Gly 510		
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Gly Ala Leu Val Phe Asn Ala Leu Trp Glu Glu Gly Ala Val Leu Ser 535 Ala Leu Lys Asn Ala Arg Phe Ala His Asn Leu Thr Ala Gln Arg Met 550 555 Glu Phe Asp Tyr Ser Thr Asn Val Trp Gly Phe Ala Phe Gly Gly Phe 565 570 Arg Thr Leu Ser Ala Glu Asn Leu Val Ala Ile Asp Gly Tyr Lys Gly 580 585 Ala Tyr Gly Gly Ala Ser Ala Gly Val Asp Ile Gln Leu Met Glu Asp 600 Phe Val Leu Gly Val Ser Gly Ala Ala Phe Leu Gly Lys Met Asp Ser 615 620 Gln Lys Phe Asp Ala Glu Val Ser Arg Lys Gly Val Val Gly Ser Val 630 635 Tyr Thr Gly Phe Leu Ala Gly Ser Trp Phe Phe Lys Gly Gln Tyr Ser 645 650 Leu Gly Glu Thr Gln Asn Asp Met Lys Thr Arg Tyr Gly Val Leu Gly 660 665 Glu Ser Ser Ala Ser Trp Thr Ser Arg Gly Val Leu Ala Asp Ala Leu 680 Val Glu Tyr Arg Ser Leu Val Gly Pro Val Arg Pro Thr Phe Tyr Ala 695 700 Leu His Phe Asn Pro Tyr Val Glu Val Ser Tyr Ala Ser Met Lys Phe 710 715 Pro Gly Phe Thr Glu Gln Gly Arg Glu Ala Arg Ser Phe Glu Asp Ala 730 Ser Leu Thr Asn Ile Thr Ile Pro Leu Gly Met Lys Phe Glu Leu Ala 745 Phe Ile Lys Gly Gln Phe Ser Glu Val Asn Ser Leu Gly Ile Ser Tyr 760 765 Ala Trp Glu Ala Tyr Arg Lys Val Glu Gly Gly Ala Val Gln Leu Leu 775 Glu Ala Gly Phe Asp Trp Glu Gly Ala Pro Met Asp Leu Pro Arg Gln - 795 Glu Leu Arg Val Ala Leu Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe 805 810 Ser Thr Val Leu Gly Leu Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr 825 Asp Ser Lys Leu Gly Tyr Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe 835 840 <210> 193 <211> 778 <212> PRT <213> Chlamydia <400> 193 Met His His His His His Gly Leu Ala Ser Cys Val Asp Leu His Ala Gly Gly Gln Ser Val Asn Glu Leu Val Tyr Val Gly Pro Gln Ala 25 Val Leu Leu Asp Gln Ile Arg Asp Leu Phe Val Gly Ser Lys Asp 40 Ser Gln Ala Glu Gly Gln Tyr Arg Leu Ile Val Gly Asp Pro Ser Ser 50

65					70					75					n Ser 80
				85					90					95	p Gln
			10	0				109	5				110	า	r Ser
		11.	5				120)				12!	5		a Phe
	13	J				135					140)			s Ala
145	•				150					155	5				e Glu 160
				165	5				170)				175	ı Gln
			180)				185	;				190)	Gly
		195	5				200					205	<u>,</u>		Ser
	210	1				215					220)			Gly
225					230					235					Val 240
			,	245					250					255	Asn
			260		Ala			265					270		
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	290				Ser	295					300				
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			340		Cys			345					350		
		355			Asn		360					365			
	3/0				Thr	375					380				
303					11e 390					395					400
				405	Phe				410					415	
			420		Ser			425					120		
		433			Phe		440					445			
	400				Gln (455					460				
400					Ala (475					400
				485	Asn (490					495	
via	TIIL.	ату	пλг	val	Glu :	rie .	ı'hr .	Asn .	Asn	Ser	Gly	Gly	Ile	Ser	Phe

500 505 Thr Gly Asn Ala Arg Ala Pro Gln Ala Leu Pro Thr Gln Glu Glu Phe 520 Pro Leu Phe Ser Lys Lys Glu Gly Arg Pro Leu Ser Ser Gly Tyr Ser 535 540 Gly Gly Gly Ala Ile Leu Gly Arg Glu Val Ala Ile Leu His Asn Ala 550 555 Ala Val Val Phe Glu Gln Asn Arg Leu Gln Cys Ser Glu Glu Glu Ala 565 570 Thr Leu Leu Gly Cys Cys Gly Gly Gly Ala Val His Gly Met Asp Ser 580 585 Thr Ser Ile Val Gly Asn Ser Ser Val Arg Phe Gly Asn Asn Tyr Ala 600 Met Gly Gln Gly Val Ser Gly Gly Ala Leu Leu Ser Lys Thr Val Gln 615 620 Leu Ala Gly Asn Gly Ser Val Asp Phe Ser Arg Asn Ile Ala Ser Leu 630 635 Gly Gly Gly Ala Leu Gln Ala Ser Glu Gly Asn Cys Glu Leu Val Asp 645 650 Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg Val Tyr Gly Gly 665 Ala Ile Ser Cys Leu Arg Gly Asp Val Val Ile Ser Gly Asn Lys Gly 680 685 Arg Val Glu Phe Lys Asp Asn Ile Ala Thr Arg Leu Tyr Val Glu Glu 695 Thr Val Glu Lys Val Glu Glu Val Glu Pro Ala Pro Glu Gln Lys Asp 710 715 Asn Asn Glu Leu Ser Phe Leu Gly Ser Val Glu Gln Ser Phe Ile Thr 725 730 Ala Ala Asn Gln Ala Leu Phe Ala Ser Glu Asp Gly Asp Leu Ser Pro 745 Glu Ser Ser Ile Ser Ser Glu Glu Leu Ala Lys Arg Arg Glu Cys Ala 755 760 Gly Gly Ala Asp Ser Ser Arg Ser Gly Cys 770 775 <210> 194 <211> 948 <212> PRT <213> Chlamydia <400> 194 Met Ala Ser Met His His His His His Val Lys Ile Glu Asn Phe Ser Gly Gln Gly Ile Phe Ser Gly Asn Lys Ala Ile Asp Asn Thr Thr Glu Gly Ser Ser Ser Lys Ser Asn Val Leu Gly Gly Ala Val Tyr Ala Lys Thr Leu Phe Asn Leu Asp Ser Gly Ser Ser Arg Arg Thr Val Thr Phe Ser Gly Asn Thr Val Ser Ser Gln Ser Thr Thr Gly Gln Val Ala 70 75 Gly Gly Ala Ile Tyr Ser Pro Thr Val Thr Ile Ala Thr Pro Val Val 90 Phe Ser Lys Asn Ser Ala Thr Asn Asn Ala Asn Asn Ala Thr Asp Thr

			100)				105	5				110)	
Glr	n Arg	J Lys 115		Thr	Phe	e Gly	7 Gly 120		ı Ile	e Gly	y Ala	Th:	Sei	Ala	a Val
Ser	Let 130		Gly	Gly	r Ala	His 135		e Leu	Glu	ı Ası	n Val		a Asp	Let	ı Gly
Ser 145	Ala	ı Ile	Gly	Leu	Val 150) Asp	Thr	Glr	Asr 155		Gli	Thr	Val	Lys 160
				165					170)				175	Arg
			180					185					190	Ala	Thr
		195	ı				200					205	ı		Thr
	210)				215					220			_	Asn
225					230					235	.				Thr 240
				245					250					255	Ile
			Asn 260					265					270		
		275	Gly				280					285			
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			Thr	325					330					335	
			Asp 340					345					350		
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			420 Asn					425					430		
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465			Ala		470					475					480
			Ala	485					490					495	
			500 Ala					505					510		
		515	Val				520					525			
	530					535	~ - y	JIU	116	nγs	540	тте	Asp	P10	АЅП

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545	5				550					555	5				Ser 560
				565					570)				575	
			580					585					590)	Leu
Thr	Leu	Asp 595		Asp	Gln	Leu	Gln 600		Gly	Thr	Ile	Ser 605		Leu	Trp
Lys	Phe 610		Ser	Tyr	Arg	Gln 615		Ala	Tyr	. Val	Prc 620		Asp	Asn	His
625	;				630					635					Val 640
				645					650					655	
			660					665					670		Ser
		675					680					685			Gly
	690					695					700				Gly
705					Met 710					715					720
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			740		Phe			745					750		
		755			Leu		760					765			
	770				His	775					780				
785					Gly 790					795					800
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			820		Ser			825					830		
		835			Phe		840					845			
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865					Ser 870					875					880
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			900		Pro			905			•		910		
		915			Gly		920					925			
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945	E €	TIIL	rue												

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				405	5				41	0					
Let	ı Ser	r Lei	ı Se:			Lvs	The	· Va			The	· T.		415	Ala
			420)		. <i></i>		425		т пе	7 1111	. цу:	430		Ala
Lys	s Glu	ı Sei	r Gly	, Gly	, Ala	Ile	Phe) Le	ı Ala	. Ser	: Ile	Pro	Thr
		43	5				44()				445	5		
	450)				455					460)			Ser
Thr	Pro	o Gli	ı Val	l Val			Ala	Lys	: Ile	e Asr	n Arg	, Phe	Phe	Ala	Ser
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			500					505	i				510		
Ile	e Glu	Asr 515	ı Ile	Leu	Asn	Val	Al.a 520		Asr	ı Glr	Asn	Thr 525		Ala	Lys
Lys	Gly 530	Gly	' Ala	Ile	Tyr	Gly 535	Lys	Lys	Ala	Lys	Leu 540	Ser	Arg	Ile	Asn
Asn	Leu	Glu	Leu	Ser	Gly			Ser	Gln	Asp			Glv	Glv	Len
545					550					555					560
				Ser 565					570					575	Ser
His	Tyr	Asn	Ser 580	Ala	Ala	Lys	Glu	Gly 585	Gly	Val	Ile	His	Ser 590	Lys	Thr
Val	Thr	Leu 595	Ser	Asn	Leu	.Lys	Ser 600	Thr	Phe	Thr	Phe	Ala 605	Asp	Asn	Thr
Val	Lys	Ala	Ile	Val	Glu	Ser		Pro	Glu	Ala	Pro	Glu	Glu	Tle	Pro
	610					615					620				
Pro 625	Val	Glu	Gly	Glu	Glu 630	Ser	Thr	Ala	Thr		Asn	Pro	Asn	Ser	
	Glu	Gly	Ser	Ser		Asn	Thr	Asn	Leu	635 Glu	Gly	Ser	Gln	Gly	640
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Thr	Ala	Asp	Thr 660	Gly	Thr	Gly	Val	Val 665	Asn	Asn	Glu	Ser	Gln 670	Asp	Thr
Ser	Asp	Thr 675	Gly	Asn	Ala	Glu	Ser 680	Gly	Glu	Gln	Leu	Gln 685	Asp	Ser	Thr
Gln	Ser	Asn	Glu	Glu	Asn	Thr		Pro	Asn	Ser	Ser	Ile	Asp	Gln	Ser
	690					695					700				
705	Glu	Asn	Thr	Asp	Glu	Ser	Ser	Asp	Ser	His	Thr	Glu	Glu	Ile	Thr
,05					710					715					720
лэр	Giu	ser	Vai	Ser 725	ser	ser	Ser	Lys	Ser 730	Gly	Ser	Ser	Thr		Gln
Asp	Gly	Gly	Ala 740	Ala	Ser	Ser	Gly	Ala	Pro	Ser	Gly	Asp		735 Ser	Ile
Ser	Ala	Asn		Cys	Leu	Ala	Lys	745 Ser	Tyr	Ala			750 Thr	Asp	Ser
Ser	Pro	755 Val	Ser	Asn	Ser	Ser	760 Gly	Ser	Asp	Val	Thr	765 Ala	Ser	Ser	Asp
	//0					775					780				
785	PLO	Asp	Ser	Ser	Ser	Ser	Gly	Asp	Ser		Gly	Asp	Ser		
	Thr	Glu	Pro	Glu	790 סום	G1	C ^	Th	m⊾	795	mı	_		_	800
			Ala	Glu 805	uta	ату	ser	ınr	Thr 810	Glu	Thr	Pro		Leu 815	Ile
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Tyr	Ile	Gly	Val 420		Trp	Ser	Arg	Ala 425		Phe	Asp	Ala	Asp	Thr	Ile	
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ouguacycyc ceuguacege agageaatti c	31
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213> Chlamydia	

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Val Pro Ser Ser Asp Pro
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 <212> DNA
<213> Chlamydia
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<213> Chlamydia
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<211> 24
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<213> Chlamydia
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Val Pro His His His His His
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 <210> 223
 <211> 30
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cagaactagt ctagaacctg taagtggtcc
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<223> Made in a lab
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Ser Thr Asp Leu
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Lys Asn Ser Ala Phe Met His Pro Val Asn Ile Ser Thr Asp Leu Ala
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Val Ile Val Gly
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 Pro Met Pro Arg
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Ser Thr Asp Leu Ala Val Ile Val Gly Lys Gly Pro Met Pro Arg Thr
Glu Ile Val Lys
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Val Ile Val Gly Lys Gly Pro Met Pro Arg Thr Glu Ile Val Lys Lys
Val Trp Glu Tyr
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Gly Pro Met Pro Arg Thr Glu Ile Val Lys Lys Val Trp Glu Tyr Ile
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Lys Lys His Asn
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 Pro Asp Ala Asn
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<223> Made in a lab
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Asn Cys Gln Asp Gln Lys Asn Lys Arg Asn Ile Leu Pro Asp Ala Asn
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Leu Ala Lys Val
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<210> 232
<211> 20
<212> PRT
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<223> Made in a lab
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Lys Asn Lys Arg Asn Ile Leu Pro Asp Ala Asn Leu Ala Lys Val Phe
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Gly Ser Ser Asp
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<211> 20
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Ile Leu Pro Asp Ala Asn Leu Ala Lys Val Phe Gly Ser Ser Asp Pro
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Ile Asp Met Phe
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Phe Gly Ser Ser Asp Pro Ile Asp Met Phe Gln Met Thr Lys Ala Leu
Ser Lys His Ile Val Lys
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<212> PRT
<213> Artificial Sequence
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Val Glu Ile Thr Gln Ala Val Pro Lys Tyr Ala Thr Val Gly Ser Pro
                                   10
Tyr Pro Val Glu
<210> 237
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Ala Val Pro Lys Tyr Ala Thr Val Gly Ser Pro Tyr Pro Val Glu Ile
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Thr Ala Thr Gly
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<210> 238
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 Arg Asp Cys Val
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Pro Tyr Pro Val Glu Ile Thr Ala Thr Gly Lys Arg Asp Cys Val Asp
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Val Ile Ile Thr
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<211> 21
<212> PRT
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<400> 240
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Gln Leu Pro Cys Glu
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<210> 241
<211> 20
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Lys Arg Asp Cys Val Asp Val Ile Ile Thr Gln Gln Leu Pro Cys Glu
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Ala Glu Phe Val
<210> 242
<211> 20
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<220>
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 Asp Val Ile Ile Thr Gln Gln Leu Pro Cys Glu Ala Glu Phe Val Arg
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 Ser Asp Pro Ala
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Thr Gln Gln Leu Pro Cys Glu Ala Glu Phe Val Arg Ser Asp Pro Ala
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Thr Thr Pro Thr
<210> 244
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Cys Glu Ala Glu Phe Val Arg Ser Asp Pro Ala Thr Thr Pro Thr Ala
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Asp Gly Lys Leu
            20
<210> 245
<211> 20
<212> PRT
<213> Artificial Sequence
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<400> 245
Val Arg Ser Asp Pro Ala Thr Thr Pro Thr Ala Asp Gly Lys Leu Val
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Trp Lys Ile Asp
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<213> Artificial Sequence
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 Ala Thr Thr Pro Thr Ala Asp Gly Lys Leu Val Trp Lys Ile Asp Arg
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 Leu Gly Gln Gly
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 <212> PRT
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Ala Asp Gly Lys Leu Val Trp Lys Ile Asp Arg Leu Gly Gln Gly Glu
 Lys Ser Lys Ile
<210> 248
<211> 20
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<223> Made in a lab
<400> 248
Val Trp Lys Ile Asp Arg Leu Gly Gln Gly Glu Lys Ser Lys Ile Thr
Val Trp Val Lys
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<223> Made in a lab
<400> 249
Arg Leu Gly Gln Gly Glu Lys Ser Lys Ile Thr Val Trp Val Lys Pro
Leu Lys Glu Gly
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<211> 20
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<212> PRT
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 Gly Glu Lys Ser Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly
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 Cys Cys Phe Thr
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Gly Glu Lys Ser Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly
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Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly
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<211> 16
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<400> 253
Gly Asp Lys Cys Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly
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<400> 254
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 Phe Gly Val Leu
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Leu Ala Asp Pro Ser Phe Lys Ile Ser Glu Ala Phe Gly Val Leu Asn
Pro Glu Gly Ser
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<210> 256
<211> 20
<212> PRT
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<210> 257
<211> 20
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<223> Made in a lab
Ala Phe Gly Val Leu Asn Pro Glu Gly Ser Leu Ala Leu Arg Ala Thr
                                     10
                                                         15
Phe Leu Ile Asp
            20
<210> 258
<211> 20
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<223> Made in a lab
 <400> 258
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                                      10
 His Gly Val Ile
             20
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                                     10
His Ala Val Ile
            20
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<211> 20
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<223> Made in a lab
<400> 260
Thr Phe Leu Ile Asp Lys His Gly Val Ile Arg His Ala Val Ile Asn
                                     10
Asp Leu Pro Leu
            20
<210> 261
<211> 20
<212> PRT
<213> Artificial Sequence
<220>
<223> Made in a lab
<400> 261
Lys His Gly Val Ile Arg His Ala Val Ile Asn Asp Leu Pro Leu Gly
                                    10
                                                         15
Arg Ser Ile Asp
<210> 262
<211> 20
<212> PRT
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<220>
 <223> Made in a lab
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 Glu Leu Arg Ile
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 <210> 263
 <211> 897
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                                                                        120
attaaggttg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc
                                                                        180
gegggetett eegeacacat tacagettee caagtgteea aaggattagg ggatgegaga
                                                                        240
actifttigteg etttagggaa tgeetttaac ggagegttige caggaacagt teaaagtgeg
                                                                        300
caaagettet teteteacat gaaagetget agteagaaaa egeaagaagg ggatgagggg
                                                                        360
ctcacagcag atctttgtgt gtctcataag cgcagagcgg ctgcggctgt ctgtagcatc
                                                                        420
atcggaggaa ttacctacct cgcgacattc ggagctatcc gtccgattct gtttgtcaac
                                                                        480
aaaatgctgg caaaaccgtt tctttcttcc caaactaaag caaatatggg atcttctgtt
                                                                        540
agctatatta tggcggctaa ccatgcagcg tctgtggtgg gtgctggact cgctatcagt
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gcgnaaagag cagattgcga agcccgctgc gctcgtattg cgagagaaga gtcgttactc
                                                                        660 ·
gaagtgccgg gagaggaaaa tgcttgcgag aagaaagtcg ctggagagaa agccaagacg
                                                                        720
ttcacgcgca tcaagtatgc actcctcact atgctcgaga agtttttgga atgcgttgcc
                                                                        780
gacgttttca aattggtgcc gctgcctatt acaatgggta ttcgtgcgat tgtggctgct
                                                                        840
ggatgtacgt tcacttctgc aattattgga ttgtgcactt tctgcgccag agcataa
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<213> Chlamydia
<220>
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Lys Ala Phe Phe Thr Gln Pro Asn Asn Lys Met Ala Arg Val Val Asn
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Lys Thr Lys Gly Val Asp Lys Thr Ile Lys Val Ala Lys Ser Ala Ala
                            40
Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
    50
                        55
                                            60
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Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg
                     70
                                         75
 Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr
 Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln
             100
                                 105
 Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser
                             120
 His Lys Arg Arg Ala Ala Ala Val Cys Ser Ile Ile Gly Gly Ile
                         135
                                             140
 Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
                     150
                                         155
 Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
                                     170
 Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
                                 185
 Val Gly Ala Gly Leu Ala Ile Ser Ala Xaa Arg Ala Asp Cys Glu Ala
                             200
Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Pro Gly
    210
                         215
                                             220
Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr
225
                    230
                                         235
Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
                245
                                     250
Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
            260
                                 265
Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile
                             280
Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala
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                                                                       120
attaaggttg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc
                                                                       180
gcgggctctt ccgcacacat tacagcttcc caagtgtcca aaggattagg ggatgcgaga
                                                                       240
actgttgtcg ctttagggaa tgcctttaac ggagcgttgc caggaacagt tcaaagtgcg
                                                                       300
caaagcttct tctctcacat gaaagctgct agtcagaaaa cgcaagaagg ggatgaggg
                                                                       360
ctcacagcag atctttgtgt gtctcataag cgcagagcgg ctgcggctgt ctgtagcatc
                                                                       420
atcggaggaa ttacctacct cgcgacattc ggagctatcc gtccgattct gtttgtcaac
                                                                       480
aaaatgctgg caaaaccgtt tctttcttcc caaactaaag caaatatggg atcttctgtt
                                                                       540
agctatatta tggcggctaa ccatgcagcg tctgtggtgg gtgctggact cgctatcagt
                                                                       600
gcgnaaagag cagattgcga agcccgctgc gctcgtattg cgagagaaga gtcgttactc
                                                                       660
gaagtgccgg gagaggaaaa tgcttgcgag aagaaagtcg ctggagagaa agccaagacg
                                                                       720
ttcacgcgca tcaagtatgc actcctcact atgctcgaga agtttttgga atgcgttgcc
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gacgttttca aattggtgcc gctgcctatt acaatgggta ttcgtgcgat tgtggctgct ggatgtacgt tcacttctgc aattattgga ttgtgcactt tctgcgccag agcataa <210> 266 <211> 298 <212> PRT <213> Chlamydia <220> <221> VARIANT <222> (1)...(298) <223> Xaa = Any Amino Acid <400> 266 Met Ala Ser Ile Cys Gly Arg Leu Gly Ser Gly Thr Gly Asn Ala Leu 10 Lys Ala Phe Phe Thr Gln Pro Asn Asn Lys Met Ala Arg Val Val Asn Lys Thr Lys Gly Met Asp Lys Thr Ile Lys Val Ala Lys Ser Ala Ala 40 Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser 55 Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg 70 75 Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr 90 Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln 105 Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser 120 His Lys Arg Arg Ala Ala Ala Val Cys Ser Ile Tie Gly Gly Ile 135 Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn 155 Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met 170 Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val 185 Val Gly Ala Gly Leu Ala Ile Ser Ala Xaa Arg Ala Asp Cys Glu Ala 200 Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Pro Gly 215 Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr 230 235 Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu 245 250 Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met 260 265 Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala 295 <210> 267 <211> 680

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<210> 268 <211> 359 <212> DNA <213> Chlamydia	
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<pre><210> 270 <211> 219 <212> DNA <213> Chlamydia</pre>	124
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<210> 271 <211> 511 <212> DNA <213> Chlamydia	

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                                                                         120
 tgttatcgat agcttggttc ccagagaact gacaagtccc gctacattga gagaatgtaa
                                                                         180
 cctgttctcc atagatagct cctcctacta cacctgaata agttggtgtt gctggagatg
                                                                        240
 atggtgcggc tgctgcggct gcttgtaggg aagcagcagc tgcagcaggt gctgaagctg
                                                                         300 - .
 ttgttgcgac tcctgtggat gaggagtttg ctttgttgtt cgagaaagag aagcctgatt
                                                                        360
tcagattaga aatatttaca gttttagcat gtaagcctcc accttctttc ccaacaaggt
                                                                        420 .
 tetetgttae agataaggag actagangea tetagtttta aagatttttt acageagata
                                                                        480 -
 cctccaccta tctctgtagc ggagttctca g
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                                                                        120
ttatactgat aagaatcttt cgattactaa catcacagga attatcgaaa ttgcaaataa
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caaagcgaca gatgttggag gtggtgctta cgtaaaagga acccttactt gtaaaaactc
                                                                        240
tcaccgtcta caatttttga aaaactcttc cgataaacaa ggtggaggaa tctacggaga
                                                                        300
agacaacatc accctatcta atttgacagg gaagactcta ttccaagaga atactgccaa
                                                                        360
aaaagagggc ggtggactct tcataaaagg tacagataaa gctcttacaa tgacaggact
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ggatagtttc tgtttaatta ataacacatc agaaaaacat ggtggtggga gcctttgtta
                                                                        480
ccaaagaaat ctctcagact tacacctctt gatgtggaaa caattccagg aatcacgcct
                                                                        540
gtacatggtg aaacagtcat tactggcaat aaatctacag gaggtaatgg tggagggc
                                                                        598
<210> 273
<211> 126
<212> DNA
<213> Chlamydia
<400> 273
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gatagetttt tattageegt ttttageate etaatgagat eteetegtte gtaacaaata
                                                                        120
cgagag
                                                                        126
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<212> DNA
<213> Chlamydia
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                                                                       120
ataaccatag ttacggggga atctctttca tggtttattt tagagctcat caacctaggc
                                                                       180
atacgcctaa aacatttcct ttgaaagttc accattcgtt ctccgataag catcctcaaa
                                                                       240
ttgctaaagc tatgtggatt acgg
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                                                                        120
 ttaaaaacttg ttctcttaaa ttaattctag tatttaagta ttcaacatag cccattatta
                                                                        180
 attgaattgg ataattttgc cttaataatt cacattcttt ttcagtaatt ttaggttcta
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 aaccgtaccg ctttttttct aaaattaatg tttcttcatt attcatttta taagccactt
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 teetttattt titgattitg tietteigtt agtaatgett eaataatagt taataattt
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 <213> Chlamydia
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 tgatgaaaac ggaaacatcc tttcgccaga aactttagca ctattaaaga atcgttacgg
                                                                        180
gttagataag cctttattca cccagtatct tatctatttg aaatgtctgc taacactaga
                                                                        240
 tttcggggaa tctcttatct acaaagatcg aaatctcagc attattgctg ccgctcttcc
                                                                        300
atcttccgct attcttggac ttgaaagctt gtgtttactc gtgccgaatt cggatcc
                                                                        357
<210> 277
<211> 505
<212> DNA
<213> Chlamydia
<400> 277
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                                                                        120
ggtaaaaatc ctaaggccat accaggatgc gacaggaaag agatatctcc attaggagct
                                                                       180
cggagacacg ctgggttgtg gccacaagaa tagtattcta gttctcgtgt tgcgtaatga
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taacaataaa tgcatagtgt tacaaacatc ccagattcag ctgtctgttg atagaagaga
                                                                       300
gcagctgttt gttgaacggc ttcttgaata gaggagagct cactcaaaaa ggtatgtaac
                                                                       360
atgtttttca ggaataagga gtaggcgcac gcattgactc ctttcccgga agcatcagca
                                                                       420
acgattagaa agagtttagc ttggggacct tcgcctataa caaagatatc aaagaaatct
                                                                       480 -
cctcctaccg taactgcagg aatat
                                                                       505
<210> 278
<211> 407
<212> DNA
<213> Chlamydia
<400> 278
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aagaaaaaca gaaggcattc tccataccaa gatttgttgc atcgacaata aaactccaat
                                                                       120
ctttggctct gctaactgga gcggtgctgg tatgattaaa aactttgaag acctattcat
                                                                       180
ccttcgccca attacagaga cacagcttca ggcctttatg gacgtctggt ctcttctaga
                                                                       240
aacaaatagc tectatetgt eeccagagag egtgettaeg geecetaete etteaagtag
                                                                       300
acctactcaa caagatacag attctgatga cgaacaaccg agtaccagcc agcaagctat
                                                                       360
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ttttttcata ctagaaaata taagcttatc ctcagaggac tcttgtgttt	agcaggetgt	180 240
ttettaatga acagetgtte etetagtega ggaaateaac eegetgatga	a gagcatctat	300
gtcttgtcta tgaatcgcat gatttgtgat tctcgtgccg aattcggatc		351
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<213> Chlamydia		
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tgattettet tetgaegaaa ttetegatge geteacaagt aaattttetg	atcccacaat	180
aaaggateta getettgatt atetaattea aatagetee tetgatggga	aacttaagtc	240
egeteteatt caggeaaage ateaactgat gagedagaat eeteaggega aegeaatgtt etgttagett cagaaacett tgettecaga geaaatacat	ttgttggagg	300
gettegetee tratattice aagtaacete atcecetet aattgegeta	atttacatca	360 120
aatgettget tettactege catcagagaa aaccgetgtt atggagttte	tagtgaatgg 4	180
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<211> 577		
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ccagettatt ctagaaaagt tgggagatca aattettggt qqaattqctq	atactattot 1	.20
tgatagtaca giccaagata tittagacaa aatcacaaca gaccciicto	taggtttgtt 1	.80
gaaagetttt aacaacttte caateactaa taaaatteaa tgcaaegggt	tattcactcc 2	40
caggaacatt gaaactttat taggaggaac tgaaatagga aaattcacag	tcacacccaa 3	00
aagetetggg ageatgttet tagteteage agatattatt geateaagaa egttgtteta getttggtae gagaaggtga ttetaageee taegegatta	tggaaggcgg 3	60
ctcatcaggc gttcctaatt tatgtagtct aagaaccaga attattaata	Caggattgac 4	20 80
tccgacaacg tattcattac gtgtaggcgg tttagaaagc ggtgtggtat	gggttaatgc 5	40
cctttctaat ggcaatgata ttttaggaat aacaaat		77
<210> 282		
<211> 607		
<212> DNA		
<213> Chlamydia		
<400> 282		
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<213> Chlamydia

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 attagttaca tgtttaaaaa ttgctagaac aatattattc ccaaccaagc tctctgcggt
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 gctgaaaaaa cctaaattca aaagaatgac tcgccgctca tcttcagaaa gacgatccga
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 cttccataat tcgatgtctt tccccatggg gatctctgta gggagccagt tatttgcgca
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 gccattcaaa taatgttccc aagcccattt gtacttaata ggaacaagtt ggttgacatc
                                                                        360
 gacctggttg cagttcacta gacgcttgct atttagatta acgcgtttct gttttccatc
                                                                        420
 taaaatatct gcttgcataa gaaccgttaa ttttattgtt aatttatatg attaattact
                                                                        480
 gacatgette acaceettet tecaaagaac agacaggtge tttetteget ettteaacaa
                                                                        540
 taatteetge egaageagae ttattettea teeaaegagg etgaatteet etettattaa
                                                                        600
 tatctac
                                                                        607
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 <212> DNA
 <213> Chlamydia
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 ttctagtaag caggaaaaaa gctcgtaacg cctcttcatc ggtggctaat gtataaaagg
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ctcgtcctga ctcatgcatt tcggcatgat ctggcccaac tgaaggataa tctaatccag
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cggaaatgga gtgagtttgt aatacttgtc catcgtcatc ttgaagaaga tacgaataaa
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atccgtggaa tactccaggt cgccctgttg caaaacgtgc tgcatgtttt cctgaagaaa
                                                                       360
tgcccagtcc tcccccttcc actccaatta attggacttt tggattcggg ataaaatgat
                                                                        420
ggaaaaatcc aatagcgttg gagccacctc cgatacatgc aatcagaata tcaggatctc
                                                                        480
ttcctgcaac tgcatggatt tgctctttca.cttcagcgct tataacagac tgaaaaaatc
                                                                        540
gaacgatatc gggataaggt aaaggtccta aggccgatcc taagcaatag tgagtaaatq
                                                                       600
agtgtgttgt tgcccaatct tgtagagctt gattaactgc atctttgagt ccacaagatc
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cttttgttac agaaacgact tcagcaccta aaaagcgcat tttctctaca tttggtttct
                                                                       720
gtcgttccac atcttttgct cccatgtata ctacacaatc taatcctaga taagcacacg
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ctgttgctgt tgctactcca tgttgtcccg cacctgtttc agctacaaca cgtgttttcc
                                                                       840
caagatattt agcaagcaaa cactgaccaa gagcattatt cagtttatgt gctcctgtat
                                                                       900
gcaaaagatc ttcgcgttta agaaatactc tagggccatc aatagctcga gcaaaattct
                                                                       960
taacttcagt cagaggagtt tgtctccccg catagttttt caaaatacaa tctagttcag
                                                                      1020
ataaaaaact ttgctgagtt ttgagaatct cccattccgc ttttagattc tgtatag
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<212> DNA
<213> Chlamydia
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aagaaaaaca gaaggcattc tccataccaa gatttgttgc atcgacaata aaactccaat
                                                                       120
ctttggctct gctaactgga gcggtgctgg tatgattaaa aactttgaag acctattcat
                                                                       180
ccttcgccca attacagaga cacagcttca ggcctttatg gacgtctggt ctcttctaga
                                                                       240
aacaaatagc tcctatctgt ccccagagag cgtgcttacg gcccctactc cttcaagtag
                                                                       300
acctactcaa caagatacag attctgatga cgaacaaccg agtaccagcc agcaagctat
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ccgtatgaga aaataggatt agggaaacaa aacgacagca aaccaca
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<210> 285
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<212> DNA
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 tgggaaaaaa gaatctgctg aattcgaaaa gatgaaaaac caattctcta acagcatggg
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 gaagatggag gaagaactgt cttctatcta ttccaagctc caagacgacg attacatgga
                                                                        240
 aggictatec gagacegeag etgeegaatt aagaaaaaaa tiegaagate tateigeaga
                                                                        300
 atacaacaca gctcaagggc agtattacca aatattaaac caaagtaatc tcaagcgcat
                                                                        360
 gcaaaagatt atggaagaag tgaaaaaagc ttctgaaact gtgcgtattc aagaaggctt
                                                                        420
 gtcagtcctt cttaacgaag atattgtctt atctatcgat agttcggcag ataaaaccga
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 tgctgttatt aaagttcttg atgattcttt tcaaaataat taacatgcga agctagccga
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 ggagtgccgt atgtctcaat ccacttattc tcttgaacaa ttagctgatt ttttgaaagt
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 cgagtttcaa ggaaatggag ctactcttct ttccggagtt gaagagatcg aggaagcaaa
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aacggcacac atcacattct tagataatga aaaatatgct aaacatttaa aatcatcgga
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 agctggcgct atcatcatat ctcgaacaca gtttcaaaaa tatcgagact tgaataaaaa
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attgcaaccg cacgcgattg aatgatacgc aagccatttc catcatggaa aagaaccctt
                                                                        180
ggacaaaaat acaaaggagg ttcactccta accagaaaaa gggagagtta gtttccatgg
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gttttcctta tatacacccg tttcacacaa ttaggagccg cgtctagtat ttggaataca
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aattgtcccc aagcgaattt tgttcctgtt tcagggattt ctcctaattg trctgtcagc
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catccgccta tggtaacgca attagctgta gtaggaagat caactccaaa caggtcatag
                                                                        420
aaatcagaaa gctcataggt gcctgcagca ataacaacat tcttgtctga gtgagcgaat
                                                                        480
tgtttaaaag atgggcgatt atgagctacc tcatcagaga ctattttaaa tagaccattt
                                                                        540
tgggtaatca atccttctat agacccatat tcatcaatga taatctcg
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<210> 287
<211> 489
<212> DNA
<213> Chlamydia
<220>
<221> misc_feature
<222> (1)...(489)
<223> n = A, T, C \text{ or } G
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acaagtagct gttatgtatg gttctagttg cttactgcgc gccgtgggcg atttagcgaa
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aaatgattct tctattcaag tacgcatcac tgcttatcgt gctgcagccg tgttggagat
                                                                       180
acaagatctt gtgcctcatt tacgagttgt agtccaaaat acacaattag atggaacgga
                                                                       240
aagaagagaa gettggagat etttatgtgt tettaetegg eeteatagtg gtgtattaae
                                                                       300
tggcatagat caagetttaa tgacetgtga gatgttaaag gaatateetg aaaagtgtae
                                                                       360
ggaagaacag attcgtacat tattggctgc agatcatcca gaagtgcagg tagctacttt
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acagatcatt ctgagaggag gtagagtatt ccggtcatct tctataatgg aatcggttct
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cgtgccgnt
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 <213> Chlamydia
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                                                                        120
 gattetetea aagatgattt etaagtgeag eagteetaaa aateeacage ggaaceeaaa
                                                                        180
 tccgagagag t
                                                                        191
 <210> 289
 <211> 515
 <212> DNA
 <213> Chlamydia
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 tgcctgcaga tgtttatgcg cgttttcaga gactacaagg caaagaggtt ttgtatattt
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gtggttctga tgaatacgga atcgcaatta cccttaatgc agagttggca ggcatggggt
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atcaagaata tgtcgacatg tatcataagc ttcataaaga taccttcaag aaattgggaa
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tctatcgaaa cttgcaggaa cgcggactgg tagagaatca ggtgaccgaa cagctgtatt
                                                                        420
ctgaggaaga agggaagttt ttagcggacc gttatgttgt aggtacttgt cccaagtgtg
                                                                        480
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<211> 522
<212> DNA
<213> Chlamydia
<400> 290
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                                                                       120
tttgatgtaa attagcgcaa ttagaggggg atgaggttac ttggaaatat aaggagcgaa
                                                                       180
gcgatgaagg agatgtattt gctctggaag caaaggtttc tgaagctaac agaacattgc
                                                                       240.
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                                                                       300
cggacttaag tttcccatca gagggagcta tttgaattag ataatcaaga gctagatcct
                                                                       360
ttattgtggg atcagaaaat ttacttgtga gcgcatcgag aatttcgtca gaagaagaat
                                                                       420
catcatcgaa cgaatttttc aatcctcgaa aatcttctcc agagacttcg gaaagatctt
                                                                       480
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                                                                       522
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<211> 1002
<212> DNA
<213> Chlamydia
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ttttttacaa accctgggaa taagttagca aagtttgtag gggcaacaaa aagtttagat
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aaatgcttta agctaagtaa ggcggtttct gactgtgtcg taggatcgct ggaagaggcg
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ggatgcacag gggacgcatt gacctccgcg agaaacgccc agggtatgtt aaaaacaact
                                                                       300
cgagaagttg ttgccttagc taatgtgctc aatggagctg ttccatctat cgttaactcg
                                                                       360
actcagaggt gttaccaata cacacgtcaa gccttcgagt taggaagcaa gacaaaagaa
                                                                       420
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agaaaaacgc ctggggagta tagtaaaatg ctattaactc gaggtgatta cctattggca
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 gcttccaggg aagcttgtac ggcagtcggt gcaacgactt actcagcgac attcggtgtt
                                                                         540
 ttacgtccgt taatgttaat caataaactc acagcaaaac cattcttaga caaagcgact
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 gtaggcaatt ttggcacggc tgttgctgga attatgacca ttaatcatat ggcaggagtt
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 gctggtgctg ttggcggaat cgcattagaa caaaagctgt tcaaacgtgc gaaggaatcc
                                                                         720
 ctatacaatg agagatgtgc cttagaaaac caacaatctc agttgagtgg ggacgtgatt
                                                                         780
 ctaagcgcgg aaagggcatt acgtaaagaa cacgttgcta ctctaaaaag aaatgtttta
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 attacagtgg cttgctccgc tgcaatttct ggagccttga cggcagcatc cgcaggaatt
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                                                                       1002
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 <211> 333
 <212> PRT
 <213> Chlamydia
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             20
                                 25
 Lys Gly Ile Tyr Cys Ile Gln Gln Phe Phe Thr Asn Pro Gly Asn Lys
                             40
 Leu Ala Lys Phe Val Gly Ala Thr Lys Ser Leu Asp Lys Cys Phe Lys
                         55
Leu Ser Lys Ala Val Ser Asp Cys Val Val Gly Ser Leu Glu Glu Ala
                     70
                                         75
Gly Cys Thr Gly Asp Ala Leu Thr Ser Ala Arg Asn Ala Gln Gly Met
                 85
                                     90
Leu Lys Thr Thr Arg Glu Val Val Ala Leu Ala Asn Val Leu Asn Gly
                                 105
Ala Val Pro Ser Ile Val Asn Ser Thr Gln Arg Cys Tyr Gln Tyr Thr
        115
                             120
Arg Gln Ala Phe Glu Leu Gly Ser Lys Thr Lys Glu Arg Lys Thr Pro
                         135
                                             140
Gly Glu Tyr Ser Lys Met Leu Leu Thr Arg Gly Asp Tyr Leu Leu Ala
                    150
                                         155
Ala Ser Arg Glu Ala Cys Thr Ala Val Gly Ala Thr Thr Tyr Ser Ala
                165
                                     170
Thr Phe Gly Val Leu Arg Pro Leu Met Leu Ile Asn Lys Leu Thr Ala
            180
Lys Pro Phe Leu Asp Lys Ala Thr Val Gly Asn Phe Gly Thr Ala Val
                                                 205
Ala Gly Ile Met Thr Ile Asn His Met Ala Gly Val Ala Gly Ala Val
                        215
                                             220
Gly Gly Ile Ala Leu Glu Gln Lys Leu Phe Lys Arg Ala Lys Glu Ser
                    230
                                        235
Leu Tyr Asn Glu Arg Cys Ala Leu Glu Asn Gln Gln Ser Gln Leu Ser
                245
                                    250
Gly Asp Val Ile Leu Ser Ala Glu Arg Ala Leu Arg Lys Glu His Val
                                265
Ala Thr Leu Lys Arg Asn Val Leu Thr Leu Leu Glu Lys Ala Leu Glu
                            280
                                                285
Leu Val Val Asp Gly Val Lys Leu Ile Pro Leu Pro Ile Thr Val Ala
    290
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295

7

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Cys Ser Ala Ala Ile Ser Gly Ala Leu Thr Ala Ala Ser Ala Gly Ile
                                          315
 Gly Leu Tyr Ser Ile Trp Gln Lys Thr Lys Ser Gly Lys
                 325
                                      330
 <210> 293
 <211> 7
 <212> DNA
 <213> Chlamydia
 <400> 293
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 <210> 294
 <211> 196
 <212> PRT
 <213> Chlamydia
 <400> 294
Thr Met Gly Ser Leu Val Gly Arg Gln Ala Pro Asp Phe Ser Gly Lys
Ala Val Val Cys Gly Glu Glu Lys Glu Ile Ser Leu Ala Asp Phe Arg
                                  25
Gly Lys Tyr Val Val Leu Phe Phe Tyr Pro Lys Asp Phe Thr Tyr Val
         35
                              40
Cys Pro Thr Glu Leu His Ala Phe Gln Asp Arg Leu Val Asp Phe Glu
                          55
Glu His Gly Ala Val Val Leu Gly Cys Ser Val Asp Asp Ile Glu Thr
                      70
His Ser Arg Trp Leu Thr Val Ala Arg Asp Ala Gly Gly Ile Glu Gly
                                      90
Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser Phe Lys Ile Ser Glu Ala
            100
Phe Gly Val Leu Asn Pro Glu Gly Ser Leu Ala Leu Arg Ala Thr Phe
                            120
Leu Ile Asp Lys His Gly Val Ile Arg His Ala Val Ile Asn Asp Leu
                        135
Pro Leu Gly Arg Ser Ile Asp Glu Glu Leu Arg Ile Leu Asp Ser Leu
145
                    150
                                         155
Ile Phe Phe Glu Asn His Gly Met Val Cys Pro Ala Asn Trp Arg Ser
                165
Gly Glu Arg Gly Met Val Pro Ser Glu Glu Gly Leu Lys Glu Tyr Phe
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185.

Gln Thr Met Asp 195

<210> 295

<211> 181

<212> PRT

<213> Chlamydia

<400> 295

Lys Gly Gly Lys Met Ser Thr Thr Ile Ser Gly Asp Ala Ser Ser Leu 5 10 15

Pro Leu Pro Thr Ala Ser Cys Val Glu Thr Lys Ser Thr Ser Ser Ser 20 25 30

Thr Lys Gly Asn Thr Cys Ser Lys Ile Leu Asp Ile Ala Leu Ala Ile 35 40 45

Val Gly Ala Leu Val Val Val Ala Gly Val Leu Ala Leu Val Leu Cys 50 55 60

Ala Ser Asn Val Ile Phe Thr Val Ile Gly Ile Pro Ala Leu Ile Ile 65 70 75 80

Gly Ser Ala Cys Val Gly Ala Gly Ile Ser Arg Leu Met Tyr Arg Ser 85 90 95

Ser Tyr Ala Ser Leu Glu Ala Lys Asn Val Leu Ala Glu Gln Arg Leu 100 105 110

Arg Asn Leu Ser Glu Glu Lys Asp Ala Leu Ala Ser Val Ser Phe Ile 115 120 125

Asn Lys Met Phe Leu Arg Gly Leu Thr Asp Asp Leu Gln Ala Leu Glu 130 135 140

Ala Lys Val Met Glu Phe Glu Ile Asp Cys Leu Asp Arg Leu Glu Lys 145 150 155 160

Asn Glu Gln Ala Leu Leu Ser Asp Val Arg Leu Val Leu Ser Ser Tyr 165 170 175

Thr Arg Trp Leu Asp 180

<210> 296

<211> 124

<212> PRT

<213> Chlamydia

<400> 296

Ile Tyr Glu Val Met Asn Met Asp Leu Glu Thr Arg Arg Ser Phe Ala 5 10 15

- Val Gln Gln Gly His Tyr Gln Asp Pro Arg Ala Ser Asp Tyr Asp Leu 20 25 30
- Pro Arg Ala Ser Asp Tyr Asp Leu Pro Arg Ser Pro Tyr Pro Thr Pro 35 40 45
- Pro Leu Pro Ser Arg Tyr Gln Leu Gln Asn Met Asp Val Glu Ala Gly 50 55 60
- Phe Arg Glu Ala Val Tyr Ala Ser Phe Val Ala Gly Met Tyr Asn Tyr 65 70 75 80
- Val Val Thr Gln Pro Gln Glu Arg Ile Pro Asn Ser Gln Gln Val Glu 85 90 95
- Gly Ile Leu Arg Asp Met Leu Thr Asn Gly Ser Gln Thr Phe Ser Asn 100 105 110
- Leu Met Gln Arg Trp Asp Arg Glu Val Asp Arg Glu
 115 120

<210> 297

<211> 488

<212> PRT

<213> Chlamydia

<400> 297

- Lys Gly Ser Leu Pro Ile Leu Gly Pro Phe Leu Asn Gly Lys Met Gly 5 10 15
- Phe Trp Arg Thr Ser Ile Met Lys Met Asn Arg Ile Trp Leu Leu Leu 20 25 30
- Leu Thr Phe Ser Ser Ala Ile His Ser Pro Val Arg Gly Glu Ser Leu 35 40 45
- Val Cys Lys Asn Ala Leu Gln Asp Leu Ser Phe Leu Glu His Leu Leu 50 55 60
- Gln Val Lys Tyr Ala Pro Lys Thr Trp Lys Glu Gln Tyr Leu Gly Trp 65 70 75 80
- Asp Leu Val Gln Ser Ser Val Ser Ala Gln Gln Lys Leu Arg Thr Gln 85 90 95
- Glu Asn Pro Ser Thr Ser Phe Cys Gln Gln Val Leu Ala Asp Phe Ile 100 105 110
- Gly Gly Leu Asn Asp Phe His Ala Gly Val Thr Phe Phe Ala Ile Glu 115 120 125
- Ser Ala Tyr Leu Pro Tyr Thr Val Gln Lys Ser Ser Asp Gly Arg Phe
 130 135 140

Tyr 145		· Val	. Asp) Ile	Met 150		Phe	Ser	Ser	Glu 155		Arg	y Val	. Gly	Asp 160
Glu	Leu	Leu	Glu	Val 165		Gly	Ala	. Pro	Val 170		Asp	Val	. Lev	175	Thr
Leu	Tyr	Gly	Ser 180		His	Lys	Gly	Thr 185		Ala	Glu	Glu	Ser 190		Ala
Leu	Arg	Thr 195		Phe	Ser	Arg	Met 200		Ser	Leu	Gly	His 205	-	Va]	Pro
Ser	Gly 210	Arg	Thr	Thr	Leu	Lys 215	Ile	Arg	Arg	Pro	Phe 220	Gly	Thr	Thr	Arg
Glu 225	Val	Arg	Val	Lys	Trp 230	Arg	Tyr	Val	Pro	Glu 235	Gly	Val	Gly	Asp	Leu 240
Ala	Thr	Ile	Ala	Pro 245	Ser	Ile	Arg	Ala	Pro 250	Gln	Leu	Gln	Lys	Ser 255	Met
Arg	Ser	Phe	Phe 260	Pro	Lys	Lys	Asp	Asp 265	Ala	Phe	His	Arg	Ser 270	Ser	Ser
Leu	Phe	Tyr 275	Ser	Pro	Met	Val	Pro 280	His	Phe	Trp	Ala	Glu 285	Leu	Arg	Asn
His	Tyr 290	Ala	Thr	Ser	Gly	Leu 295	Lys	Ser	Gly	Tyr	Asn 300	Ile	Gly	Ser	Thr
Asp 305	Gly	Phe	Leu	Pro	Val 310	Ile	Gly	Pro	Val	Ile 315	Trp	Glu	Ser	Glu	Gly 320
Leu	Phe	Arg	Ala	Tyr 325	Ile	Ser	Ser	Val	Thr 330	Asp	Gly	Asp	Gly	Lys 335	Ser
His	Lys	Val	Gly 340	Phe	Leu	Arg	Ile	Pro 345	Thr	Tyr	Ser	Trp	Gln 350	Asp	Met
Glu	Asp	Phe 355	Asp	Pro	Ser	Gly	Pro 360	Pro	Pro	Trp	Glu	Glu 365	Phe	Ala	Lys
Ile	Ile 370	Gln	Val	Phe	Ser	Ser 375	Asn	Thr	Glu	Ala	Leu 380	Ile	Ile	Asp	Gln
Thr 385	Asn	Asn	Pro	Gly	Gly 390	Ser	Val	Leu	Tyr	Leu 395	Tyr	Ala	Leu	Leu	Ser 400
1et	Leu	Thr	Asp	Arg 405	Pro	Leu	Glu	Leu	Pro 410	Lys	His	Arg	Met	Ile 415	Leu
Thr	Gln	Asp	Glu 420	Val	Val	Asp	Ala	Leu 425	Asp	Trp	Leu	Thr	Leu 430	Leu	Glu

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Asn Val Asp Thr Asn Val Glu Ser Arg Leu Ala Leu Gly Asp Asn Met 435 440 445

Glu Gly Tyr Thr Val Asp Leu Gln Val Ala Glu Tyr Leu Lys Ser Phe 450 455 460

Gly Arg Gln Val Leu Asn Cys Trp Ser Lys Gly Asp Ile Glu Leu Ser 465 470 475 480

Thr Pro Ile Pro Leu Phe Gly Phe 485

<210> 298

<211> 140

<212> PRT

<213> Chlamydia

<400> 298

Arg Ile Asp Ile Ser Ser Val Thr Phe Phe Ile Gly Ile Leu Leu Ala 5 10 15

Val Asn Ala Leu Thr Tyr Ser His Val Leu Arg Asp Leu Ser Val Ser 20 25 30

Met Asp Ala Leu Phe Ser Arg Asn Thr Leu Ala Val Leu Leu Gly Leu 35 40 45

Val Ser Ser Val Leu Asp Asn Val Pro Leu Val Ala Ala Thr Ile Gly 50 55 60

Met Tyr Asp Leu Pro Met Asn Asp Pro Leu Trp Lys Leu Ile Ala Tyr 65 70 75 80

Thr Ala Gly Thr Gly Gly Ser Ile Leu Ile Ile Gly Ser Ala Ala Gly 85 90 95

Val Ala Tyr Met Gly Met Glu Lys Val Ser Phe Gly Trp Tyr Val Lys 100 105 110

His Ala Ser Trp Ile Ala Leu Ala Ser Tyr Phe Gly Gly Leu Ala Val 115 120 125

Tyr Phe Leu Met Glu Asn Cys Val Asn Leu Phe Val

<210> 299

<211> 361

<212> PRT

<213> Chlamydia

<400> 299

His Gln Glu Ile Ala Asp Ser Pro Leu Val Lys Lys Ala Glu Gln 5 10 15

- Ile Asn Gln Ala Gln Gln Asp Ile Gln Thr Ile Thr Pro Ser Gly Leu 20 25 30
- Asp Ile Pro Ile Val Gly Pro Ser Gly Ser Ala Ala Ser Ala Gly Ser 35 40 45
- Ala Ala Gly Ala Leu Lys Ser Ser Asn Asn Ser Gly Arg Ile Ser Leu 50 55 60
- Leu Leu Asp Asp Val Asp Asn Glu Met Ala Ala Ile Ala Met Gln Gly 65 70 75 80
- Phe Arg Ser Met Ile Glu Gln Phe Asn Val Asn Asn Pro Ala Thr Ala 85 90 95
- Lys Glu Leu Gln Ala Met Glu Ala Gln Leu Thr Ala Met Ser Asp Gln 100 105 110
- Leu Val Gly Ala Asp Gly Glu Leu Pro Ala Glu Ile Gln Ala Ile Lys
 115 120 125
- Asp Ala Leu Ala Gln Ala Leu Lys Gln Pro Ser Ala Asp Gly Leu Ala 130 135 140
- Thr Ala Met Gly Gln Val Ala Phe Ala Ala Lys Val Gly Gly 145 150 155 160
- Ser Ala Gly Thr Ala Gly Thr Val Gln Met Asn Val Lys Gln Leu Tyr 165 170 175
- Lys Thr Ala Phe Ser Ser Thr Ser Ser Ser Ser Tyr Ala Ala Ala Leu 180 185 190
- Ser Asp Gly Tyr Ser Ala Tyr Lys Thr Leu Asn Ser Leu Tyr Ser Glu 195 200 205
- Ser Arg Ser Gly Val Gln Ser Ala Ile Ser Gln Thr Ala Asn Pro Ala 210 215 220
- Leu Ser Arg Ser Val Ser Arg Ser Gly Ile Glu Ser Gln Gly Arg Ser 225 230 235 240
- Ala Asp Ala Ser Gln Arg Ala Ala Glu Thr Ile Val Arg Asp Ser Gln 245 250 255
- Thr Leu Gly Asp Val Tyr Ser Arg Leu Gln Val Leu Asp Ser Leu Met 260 265 270
- Ser Thr Ile Val Ser Asn Pro Gln Ala Asn Gln Glu Glu Ile Met Gln 275 280 285
- Lys Leu Thr Ala Ser Ile Ser Lys Ala Pro Gln Phe Gly Tyr Pro Ala 290 295 300

Val Gln Asn Ser Val Asp Ser Leu Gln Lys Phe Ala Ala Gln Leu Glu 305 310 315 320

Arg Glu Phe Val Asp Gly Glu Arg Ser Leu Ala Glu Ser Gln Glu Asn 325 330 335

Ala Phe Arg Lys Gln Pro Ala Phe Ile Gln Gln Val Leu Val Asn Ile 340 345 350

Ala Ser Leu Phe Ser Gly Tyr Leu Ser 355 360

<210> 300

<211> 207

<212> PRT

<213> Chlamydia

<400> 300

Ser Ser Lys Ile Val Ser Leu Cys Glu Gly Ala Val Ala Asp Ala Arg
5 10 15

Met Cys Lys Ala Glu Leu Ile Lys Lys Glu Ala Asp Ala Tyr Leu Phe 20 25 30

Cys Glu Lys Ser Gly Ile Tyr Leu Thr Lys Lys Glu Gly Ile Leu 1le 35 40 45

Pro Ser Ala Gly Ile Asp Glu Ser Asn Thr Asp Gln Pro Phe Val Leu 50 55 60

Tyr Pro Lys Asp Ile Leu Gly Ser Cys Asn Arg Ile Gly Glu Trp Leu 65 70 75 80

Arg Asn Tyr Phe Arg Val Lys Glu Leu Gly Val Ile Ile Thr Asp Ser

His Thr Thr Pro Met Arg Arg Gly Val Leu Gly Ile Gly Leu Cys Trp
100 105 110

Tyr Gly Phe Ser Pro Leu His Asn Tyr Ile Gly Ser Leu Asp Cys Phe 115 120 125

Gly Arg Pro Leu Gln Met Thr Gln Ser Asn Leu Val Asp Ala Leu Ala 130 135 140

Val Ala Ala Val Val Cys Met Gly Glu Gly Asn Glu Gln Thr Pro Leu 145 150 155 160

Ala Val Ile Glu Gln Ala Pro Asn Met Val Tyr His Ser Tyr Pro Thr 165 170 175

Ser Arg Glu Glu Tyr Cys Ser Leu Arg Ile Asp Glu Thr Glu Asp Leu 180 185 190 Tyr Gly Pro Phe Leu Gln Ala Val Thr Trp Ser Gln Glu Lys Lys 195 200 205

<210> 301

<211> 183

<212> PRT

<213> Chlamydia

<400> 301

Ile Pro Pro Ala Pro Arg Gly His Pro Gln Ile Glu Val Thr Phe Asp
5 10 15

Ile Asp Ala Asn Gly Ile Leu His Val Ser Ala Lys Asp Ala Ala Ser 20 25 30

Gly Arg Glu Gln Lys Ile Arg Ile Glu Ala Ser Ser Gly Leu Lys Glu 35 40 45

Asp Glu Ile Gln Gln Met Ile Arg Asp Ala Glu Leu His Lys Glu Glu 50 55 60

Asp Lys Gln Arg Lys Glu Ala Ser Asp Val Lys Asn Glu Ala Asp Gly 65 70 75 80

Met Ile Phe Arg Ala Glu Lys Ala Val Lys Asp Tyr His Asp Lys Ile 85 90 95

Prc Ala Glu Leu Val Lys Glu Ile Glu Glu His Ile Glu Lys Val Arg

Gln Ala Ile Lys Glu Asp Ala Ser Thr Thr Ala Ile Lys Ala Ala Ser 115 120 125

Asp Glu Leu Ser Thr Arg Met Gln Lys Ile Gly Glu Ala Met Gln Ala 130 135 140

Gln Ser Ala Ser Ala Ala Ser Ser Ala Ala Asn Ala Gln Gly Gly
145 150 155 160

Pro Asn Ile Asn Ser Glu Asp Leu Lys Lys His Ser Phe Ser Thr Arg 165 170 175

Pro Pro Ala Gly Gly Ser Ala 180

<210> 302

<211> 232

<212> PRT

<213> Chlamydia

<400> 302

Met Thr Lys His Gly Lys Arg Ile Arg Gly Ile Gln Glu Thr Tyr Asp
5 10 15

- Leu Ala Lys Ser Tyr Ser Leu Gly Glu Ala Ile Asp Ile Leu Lys Gln 20 25 30
- Cys Pro Thr Val Arg Phe Asp Gln Thr Val Asp Val Ser Val Lys Leu 35 40 45
- Gly Ile Asp Pro Arg Lys Ser Asp Gln Gln Ile Arg Gly Ser Val Ser 50 55 60
- Leu Pro His Gly Thr Gly Lys Val Leu Arg Ile Leu Val Phe Ala Ala 65 70 75 80
- Gly Asp Lys Ala Ala Glu Ala Ile Glu Ala Gly Ala Asp Phe Val Gly 85 90 95
- Ser Asp Asp Leu Val Glu Lys Ile Lys Gly Gly Trp Val Asp Phe Asp 100 105 110
- Val Ala Val Ala Thr Pro Asp Met Met Arg Glu Val Gly Lys Leu Gly 115 120 125
- Lys Val Leu Gly Pro Arg Asn Leu Met Pro Thr Pro Lys Ala Gly Thr 130 135 140
- Val Thr Thr Asp Val Val Lys Thr Ile Ala Glu Leu Arg Lys Gly Lys 145 150 155 160
- Ile Glu Phe Lys Ala Asp Arg Ala Gly Val Cys Asn Val Gly Val Ala 165 170 175
- Lys Leu Ser Phe Asp Ser Ala Gln Ile Lys Glu Asn Val Glu Ala Leu 180 185 190
- Cys Ala Ala Leu Val Lys Ala Lys Pro Ala Thr Ala Lys Gly Gln Tyr 195 200 205
- Leu Val Asn Phe Thr Ile Ser Ser Thr Met Gly Pro Gly Val Thr Val 210 215 220
- Asp Thr Arg Glu Leu Ile Ala Leu 225 230
- <210> 303
- <211> 238
- <212> PRT
- <213> chlamydia
- <400> 303
- Ile Asn Ser Lys Leu Glu Thr Lys Asn Leu Ile Tyr Leu Lys Leu Lys
 5 10 15
- Ile Lys Lys Ser Phe Lys Met Gly Asn Ser Gly Phe Tyr Leu Tyr Asn 20 25 30





Thr Gln Asn Cys Val Phe Ala Asp Asn Ile Lys Val Gly Gln Met Thr 35 40 45

Glu Pro Leu Lys Asp Gln Gln Ile Ile Leu Gly Thr Thr Ser Thr Pro 50 55 60

Val Ala Ala Lys Met Thr Ala Ser Asp Gly Ile Ser Leu Thr Val Ser 65 70 75 80

Asn Asn Pro Ser Thr Asn Ala Ser Ile Thr Ile Gly Leu Asp Ala Glu 85 90 95

Lys Ala Tyr Gln Leu Ile Leu Glu Lys Leu Gly Asp Gln Ile Leu Gly
100 105 110

Gly Ile Ala Asp Thr Ile Val Asp Ser Thr Val Gln Asp Ile Leu Asp 115 120 125

Lys Ile Thr Thr Asp Pro Ser Leu Gly Leu Leu Lys Ala Phe Asn Asn 130 135 140

Phe Pro Ile Thr Asn Lys Ile Gln Cys Asn Gly Leu Phe Thr Pro Arg 145 150 155 160

Asn Ile Glu Thr Leu Leu Gly Gly Thr Glu Ile Gly Lys Phe Thr Val 165 170 175

Thr Pro Lys Ser Ser Gly Ser Met Phe Leu Val Ser Ala Asp Ile Ile
180 185 190

Ala Ser Arg Met Glu Gly Gly Val Val Leu Ala Leu Val Arg Glu Gly 195 200 205

Asp Ser Lys Pro Tyr Ala Ile Ser Tyr Gly Tyr Ser Ser Gly Val Pro 210 215 220

Asn Leu Cys Ser Leu Arg Thr Arg Ile Ile Asn Thr Gly Leu 225 230 235